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Attn: Section 8(e) Coordinator (CAP Agreement)

Re: CAP Agreement Identification No. 8ECAP-0110

Dear Sir or Madam:

Union Carbide Corporation ("Union Carbide") herewith submits the following report pursuant to the terms of the TSCA §8(e) Compliance Audit Program and Union Carbide's CAP Agreement dated August 14, 1991 (8ECAP-0110). This report describes a study on the hematologic toxicity of ethylene glycol monobutyl ether (EGBE; CASRN 111-76-2).

"Studies on the Hematologic Toxicity of Ethylene Glycol Monobutyl Ether (EGBE)", The George Washington University (to CMA), Contract No. GE-30.0-GWU, September 30, 1990.

A complete summary of this report is attached.

Previous TSCA Section 8(e) or "FYI" Submission(s) related to this substance are:

(None)

Previous PMN submissions related to this substance are: (None)

egbe

9/13/95

This information is submitted in light of EPA's current guidance. Union Carbide does not necessarily agree that this information reasonably supports the conclusion that the subject chemical presents a substantial risk of injury to health or the environment.

In the attached report the term "CONFIDENTIAL" may appear. This precautionary statement was for internal use at the time of issuance of the report. Confidentiality is hereby waived for purposes of the needs of the Agency in assessing health and safety information. The Agency is advised, however, that the publication rights to the contained information are the property of Union Carbide.

Yours truly,

William C. Kuryla, Ph.D.
Associate Director
Product Safety
(203/794-5230)

WCK/cr
Attachment (3 copies of cover letter, summary, and report)

egbe

SUMMARY

Studies on the Hematologic Toxicity of Ethylene Glycol Monobutyl Ether (EGBE)

Final Report for Contract No. GE-30.0-GWU for

The Chemical Manufacture Association
2501 M St. NW
Washington, DC 20037

Submitted by:

The George Washington University

for:

Joseph Kurantsin-Mills, Ph.D.
and
Lawrence S. Lessin, M.D.

Division of Hematology and Oncology
Department of Medicine
(202) 994-2867

Administrative Address:

The George Washington University
Office of Sponsored Research
Rice Hall - Sixth Floor
Washington, DC 20006
(202) 994-6255

Date: September 27, 1990

Date September 27, 1990

SUMMARY

Studies were conducted to investigate some of the mechanistic processes underlying the hematologic toxicity of ethylene glycol monobutyl ether (EGBE) in the rat. Sprague-Dawley rats, (145-350gm) were administered EGBE by gavage (50-500mg/kg BW), and blood was sampled after 0.5hr, 2hr, and 4hr for the determination of complete physical characteristics of erythrocytes, including peripheral cellular kinetics and frequency distribution. The erythrocyte shape, buoyant density, energy content, propensity to generate lipid peroxides in vitro, and dose and time-dependent lysis were determined. Whole blood viscosity was evaluated over a wide range of shear rates. Plasma and urine hemoglobin concentrations were measured by spectrophotometry based on the absorption properties of hemoglobin at 415nm and 540nm. The deformability of the red cells through model flow cylindrical pores that simulated microvessels was estimated as the relative resistance of a cell in a single pore.

Pairwise comparisons among the group means indicated that EGBE-treated rats did not express a clear dose- and time-dependent relationship in the circulating erythrocyte population. Scanning electron microscopy revealed that the red cell shape was transformed from discocyte to spherocyte thereby significantly increasing the cell volume (MCV) and decreasing the cell hemoglobin concentration (MCHC). The concomitant increase in the MCV and decrease in the MCHC was confirmed by direct measurement using a laser flow cytometer, and computer analysis of the frequency distribution of these indices. The median buoyant density of the red cells also decreased as a function of EGBE concentration, whereas the mean adenosine triphosphate concentration was within one standard deviation of normal, and not significantly different among the treated animals. Measurements of plasma and urine hemoglobin using the absorption peaks at 415nm and 540nm revealed very low levels of hemolysis. The in vitro generation of malonyldialdehyde (MDA) which reflects the degree of lipid peroxidation decreased as a function of EGBE dose. This observation suggests that the hepatic metabolic by-products of EGBE, such as butoxyacetic acid (BAA) may be acting as free radical scavengers. Whole blood viscosity increased at the shear rates 5.75-230/s for doses 50 and 100 mg/kg, and then decreased thereafter due to hemolysis. In vitro simulation of shear-dependent hemolysis using the viscometer at shear forces of 0.621 to 13.20 dynes/cm' indicated that the red cells of EGBE-treated rats hemolyzed more than the controls. Filtration studies showed that the deformability of the red blood cells of EGBE-treated rats was significantly less than the control cells due to the decreased surface area to volume ratio. These results demonstrate that hematologic toxicity of EGBE has multiple underlying mechanisms involving biophysical and biochemical changes of the blood cells. The membrane and cellular changes in the red cell due to EGBE and/or its metabolites would alter its rheological properties and thereby compromise its transit through the very narrow vessels of the microcirculation resulting in hemolysis.

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ACKNOWLEDGEMENTS

This research work was supported by the Chemical Manufacturers Association, Washington DC. We appreciate the periodic lively and useful discussions we shared with Dr. Carol R. Stack (Associate Director, Biomedical and Environmental Special Programs, CMA, Washington, DC.), Dr. Tipton R. Tyler (Assistant Corporate Director of Applied Toxicology, Health, Safety and Environmental Affairs Department, Union Carbide Corporation, Danbury, CT), Dr. Ralph Gingell (Senior Toxicologist, Health, Safety and Environment, Shell Oil Company, Houston, TX), and other members of the Glycol Ether Study Panel of the CMA. Several useful and interesting ideas were generated during these discussions, some of which are incorporated in the present study. These include the hematological indices by the laser-light scattering technique, analysis of plasma and urine hemoglobin concentrations to determine the extent of EGBE-related hemolysis, and viscosity. Unfortunately, all of the ideas discussed could not be investigated at this time because we preferred to restrict our present efforts to the specifications of the contract. However, we have suggested some new experiments for future research in the discussion part of this report. These include specific areas we would like to see investigated so that the mechanisms underlying the variations in species responses to the hematotoxicity of ethylene glycol monobutyl ether can be elucidated.

We acknowledge the expert assistance of our long time collaborator Dr. Richard Entsuah of the University of Illinois at Chicago (currently at Wyeth Pharmaceutical, Philadelphia) with all the statistical analyses of the data. His role as an unpaid consultant is most appreciated. Furthermore, his assistance with computer data analyses and graphics during the final stages of this study was most helpful and is very much appreciated.

We also acknowledge the excellent technical assistance of Kirvin L. Hodge of the Division of Hematology and Oncology, Department of Medicine, The George Washington University Medical Center.

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SUMMARY

Studies were conducted to investigate some of the mechanistic processes underlying the hematologic toxicity of ethylene glycol monobutyl ether (EGBE) in the rat. Sprague-Dawley rats, (145-350gm) were administered EGBE by gavage (50-500mg/kg BW), and blood was sampled after 0.5hr, 2hr, and 4hr for the determination of complete physical characteristics of erythrocytes, including peripheral cellular kinetics and frequency distribution. The erythrocyte shape, buoyant density, energy content, propensity to generate lipid peroxides in vitro, and dose and time-dependent lysis were determined. Whole blood viscosity was evaluated over a wide range of shear rates. Plasma and urine hemoglobin concentrations were measured by spectrophotometry based on the absorption properties of hemoglobin at 415nm and 540nm. The deformability of the red cells through model flow cylindrical pores that simulated microvessels was estimated as the relative resistance of a cell in a single pore.

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I. LITERATURE REVIEW

Evidence for hematotoxicity of Ethylene Glycol Monobutyl Ether

Glycol ethers belong to a class of solvents with widespread industrial applications because of their dual solubilizing characteristics of alcohols and ethers. Because of these unique solvent characteristics and other physical and chemical properties, glycol ethers are used as (a) resin solvent in surface coatings and inks; (b) ingredients in hydraulic brake fluids; (c) dye solvents in textile and leather applications; (d) ingredients in aqueous-based cleaning agents, and (e) paints (Rowe and Wolfe, 1982). The use of glycol ethers in paints, lacquers, dyes, inks and household cleaning agents has generated considerable interest in their toxicology. The Consumer Product Safety Commission (1977) listed 742 consumer products containing ethylene glycol monobutyl ether (EGBE) at an average concentration of 2.8%.

In 1943, Werner et al. first showed that EGBE as well as other monoalkyl ethers produced hemoglobinuria in mice during an acute toxicologic study. Since that time, from 1943 through 1988, several studies have been published to define the hemotoxicity of EGBE. These studies are summarized below. The experiments described in these studies were of acute and subchronic design and performed with various species including mice, rats, guinea pigs, rabbits, dogs, monkeys and man. Exposures were largely inhalational, but also included transdermal, oral and parenteral administration. Dose, duration and frequency of exposures have varied. Hemolysis and some other hematological parameters were measured as a function of time. The salient aspects of these studies are summarized in Table 1. The studies conducted with EGBE are complicated because of the different experimental protocols applied to different species by different investigators. Nevertheless the following is apparent:

i. A substantial species variations exists in susceptibility to to the hemolytic effects of EGBE, with the rat being the most susceptible and man the most resistant.

ii. In an experiment with humans no hemolysis was demonstrated after breathing up to 195 ppm for 8 hrs.

iii. In susceptible species, the hemolytic effect of EGBE is dose-dependent with a demonstrable hemolytic threshold.

iv. The studies showed no irrevocable evidence of toxicity to to hematopoiesis or circulating leukocytes or platelets.

v. In studies where the hemolytic sequence was monitored hemolysis occurred early after initiation of EGBE exposure and was accompanied by rise in reticulocyte count and mean corpuscular volume (MCV). However, the circulating RBC and hemoglobin levels recovered after 1-2 weeks despite continued exposure. This suggests that older RBCs may be selectively hemolyzed while younger cells may be resistant.

Hemolysis and RBC alterations in rodents following exposure to EGBE.

Published toxicologic studies indicate that the rat is the most susceptible species to EGBE-induced hemolysis. Other species studied in acute and subacute experiments include mice, guinea pigs and rabbits. In the rat inhalational, oral, transdermal and parenteral administration to EGBE produced hemolysis, indicating absorption by all routes. The well known hemolytic vulnerability of rat red cells make these cells particularly good model for investigating the mechanisms of hemolysis.

In the rabbit, subchronic inhalational exposures up to 220 ppm produced minimal effects on red blood cells (RBCs) while subchronic dermal applications were hemolytic at relatively high concentrations, indicating low susceptibility of rabbit RBCs to EGBE associated-hemolysis (Carpenter et al., 1956; Homan et al., 1979; Tyl et al., 1983). In a single subchronic study, guinea pigs showed no hemolysis after six weeks of 5 hour daily exposure up to 484 ppm, a rather striking resistance. In contrast, mice hemolyzed in acute and variable subchronic exposures up to 90 days at inhaled concentrations of 112 ppm EGBE (Carpenter et al., 1956).

In other studies with rats and mice in which the homologues ethylene glycol monomethyl ether (EGME) and propylene glycol monomethyl ether were administered by inhalation, significant differences were noted in the biological activities of these structurally similar compounds. Thus, exposure of high concentration of EGME vapors (1000 ppm) to the rodents resulted in markedly reduced bone marrow cellularity (both myeloid and erythroid elements); severe degeneration of germinal epithelium of the testes; severe lymphoid depletion in cortical thymus and reduced numbers of lymphoid cells in the spleen and mesenteric lymph nodes. Thus the susceptibility ranking among various rodent species is that rats and mice are most susceptible and rabbits and guinea pigs are least susceptible (Carpenter et al., 1975abc, Lessin, 1985).

In the studies of Carpenter, Werner, Dodd and Tyl (and respective colleagues) both rats and mice exhibited crude hemolytic dose-response relationships to EGBE, as did rabbits to dermal and inhalation exposure (Lessin, 1985). Furthermore, in all these studies (Miller et al., 1981, 1983; Dodd et al., 1983; Miller et al., 1984; Grant et al., 1985; and Bartnik et al., 1987), it is apparent that the susceptibility of a test animal is also dependent on the chemical species of the glycol ether and its eventual metabolic by-products generated by hepatic enzymatic oxidation.

Hemolysis in dogs and monkeys exposed to EGBE

The two studies of EGBE toxicity in dogs were published by Werner and associates in 1943 and Carpenter and co-workers in 1956. Werner exposed two dogs subchronically to inhalation of EGBE at 415 ppm for 7 hours a day, 5 days per week for 12 weeks. At this relatively high concentration, a 10% decrease in hematocrit was noted at week 10 associated with slight change in RBC osmotic fragility; no reticulocyte response was noted. Carpenter and co-workers (1975c) exposed three separate pairs of dogs to subchronic inhalational doses of 385, 200 and 100 ppm for up to 90 days. A single female dog died 13 hours after the study began at 617 ppm and no mention was made of hemolysis in that animal. All the other dogs showed transient increase in RBC osmotic fragility, but only the dog exposed at 100 ppm for 90 days developed anemia. Carpenter's subchronic EGBE inhalational experiments on monkeys are difficult to interpret, because the test animals had severe tuberculosis infections which causes anemia. Monkeys exposed at 100 ppm for 90 days showed increased RBC osmotic fragility but only transient minor decrease in red blood cell count. One animal exposed to EGBE at

210 ppm for 30 days manifested a 50% decrease in hemoglobin by day 30, but autopsy shortly thereafter showed disseminated tuberculosis, a disease that could easily cause this degree of anemia. Thus, although dogs appear to be susceptible to EGBE associated hemolysis after subchronic vapor exposure, no dose-response relation has been established. The data obtained on the monkeys are not easily interpretable. Further studies may be warranted.

Relative resistance of human RBCs to EGBE associated hemolysis

Carpenter (1956), studied the effects of EGBE vapor inhalation in human subjects exposed to concentrations of 113 ppm for 4 hours and 195 ppm for 8 hours. During these exposures, ocular, mucous membrane, gastrointestinal and neurologic symptoms were noted. The toxic effects were sufficiently adverse for the investigators to consider that exposure to higher doses would be intolerable. Hematologic studies on the test subjects showed no evidence of hemolysis. Rats simultaneously exposed in the same chambers with the human subjects did exhibit extensive hemolysis. All human subjects excreted butoxyacetic acid in their urine indicating that they had absorbed and metabolized EGBE.

A recent industrial hygiene survey has been performed to characterize the exposure of shipyard painters to 2-ethoxyethanol and 2-methoxyethanol. One hundred and two samples were taken over six workshifts. The time-weighted average of airborne exposure to 2-ethoxyethanol ranged from 0-80.5 mg/m^3 with a mean of 9.9 mg/m^3 and a median of 4.4 mg/m^3. The equivalent values for 2-methoxy- ethanol were 0-17.7 mg/m^3, 2.6 mg/m^3, and 1.6 mg/m^3 respectively (Sparer et al., 1988). In the same study, the painters were found to have an increased prevalence of oligospermia and azoospermia, and an increased odds ratio for a lower sperm count per ejaculate, while smoking was controlled (Welch et al., 1988a). The investigators also evaluated the hematologic effects of exposure to the ethylene glycol ethers, and found that even though the average values of all variables were comparable between the control group and the painters, a significant proportion of the painters were anemic (10%) and granulocytopenic (5%). None of the controls were affected (Welch et al., 1988b). Recently, Groseneken et al. (1986) have repeated the human experiments by exposing ten healthy male volunteers (aged 19-28) to ethylene monoethyl ether (EGEE) at rest and under physical workload (bicycle ergometer). The exposure concentrations were 10 mg/m^3, 20 mg/m^3 and 40mg/m^3 and were maintained throughout the exposure period. These concentrations are within the currently accepted threshold limit value of EGEE. Retention of EGEE by the subjects was high (64 % in resting condition) and increased as a function of physical workload. Individual uptake was governed by pulmonary ventilation and cardiac output. Respiratory elimination of non-metabolized EGEE declined rapidly during the first minutes after cessation of exposure, and thereafter very slowly. The lungs also accounted for $\leq$ 0.4 % of the total body uptake.

Variations in Species Responses to EGBE-associated hemolysis

From the foregoing, it is evident that marked species differences exist in susceptibility to EGBE related-hemolysis. Clearly, rats and mice are particularly susceptible and man is not. Figure 1 shows the hemolytic thresholds (i.e. minimum levels of effect) for the species studied. None has been established for man, rabbits or guinea pigs for inhalational exposures, and these three species have shown resistance to hemolysis at subchronic and acute exposure levels ranging from about 200 to 500 ppm. Dogs show intermediate susceptibility, and no conclusions can be drawn regarding the limited nonhuman primate experiments.

Metabolism and Mechanism of Hematotoxicity of Glycol Ethers.

Carpenter et al., (1956) demonstrated the presence of butoxyacetic acid in the urine of animals given EGBE. This observation has been confirmed by subsequent studies by Jonsson and Steen (1978) who employed mass spectrometry and organic synthesis to confirm the identity of the metabolite found in the urine of the rats exposed to n-butoxyethanol. Following these initial reports, other investigators have reported the metabolism of other glycol ethers. Miller et al., (1983, 1984) utilized ^{14}C-EGME and ^{14}C-PGME to investigate the metabolism of these compounds in the rat. Methoxyacetic acid was identified as the primary urinary metabolite of EGME comprising of 80-90 % of the total ^{14}C in urine. PGMG, propylene glycol (1,2-propanediol), and the sulfate and glucuronide conjugates of PGME were also found in the urine of rats given PGME. Cheever et al. (1984) have also employed ^{14}C-2-ethoxyethanol to identify its major pathway of biotransformation in the rat. Ethoxy-acetic acid and N-ethoxyacetyl glycine representing 73% to 76 % of the administered dose were found in the urine. On the other hand, rats administered EGBE excreted 2-butoxyacetic acid as $\geq$ 75% of the ^{14}C excreted in the urine. The second major metabolites in the urine was the glucuronide conjugate (21%) of EGBE. The sulfate conjugate accounted for about 2.7% of the total ^{14}C (Ghanayem et al., 1987). Employing prior treatment of rats with inhibitors of alcohol and aldehyde dehydrogenases (pyrazole and cyanamide respectively), Ghanayem et al., (1987) demonstrated that the animals were protected against 2-butoxyethanol hematotoxicity. Furthermore, the inhibition of 2-butoxyethanol metabolism to butoxyacetate by both inhibitors was accompanied by increased metabolism of the alcohol to the glucuronide and sulfate conjugates as determined by quantitative high performance chromatographic analysis of the metabolites in the urine.

These metabolic and biotransformation studies of glycol ethers have strongly suggested that the active hematotoxic compound is the carboxylic acid metabolite. A recent report in which the butoxy-acetic acid and not EGBE (or 2-BE) caused a concentration- and time-dependent swelling of red blood cells following hemolysis, support this suggestion (Ghanayem et al., 1988). However, the mechanism of the hematotoxicity is still unknown, and would require further detailed analysis using a variety of techniques.

Biophysical and Biochemical Aspects of Hemolysis

A variety of biophysical and biochemical conditions result in the lysis of erythrocytes. Hemolysis may be defined as the premature destruction of red blood cells. Erythrocyte injury may be caused by several mechanisms that ultimately result in two possible pathophysiological events. The first is the disruption of the cell membrane during circulation and the release of hemoglobin. This is also called intravascular hemolysis, an example of which is mechanical hemolytic anemia that results from a prosthetic heart valve. The second is the destruction of red cells in the tissue macrophage (reticuloendothelial) system, also known as extravascular hemolysis, and typified by hereditary spherocytosis. In several hemolytic disorders, both mechanisms may operate and therefore it would be prudent to make the precise diagnosis. Hemolysis can be broadly classified as intrinsic to the red cells or extrinsic to the cells (Lessin and Bessis, 1983). Intrinsic hemolysis arises primarily from inherited disorders of the red cell membrane proteins, of hemoglobin structure, of hemoglobin chain synthesis, or of enzyme deficiency.

Extrinsic injury to erythrocytes include physical and chemical factors. An example of a physical factor is heat, which denatures the proteins of the membrane, alters the lipid components and disrupts the cell. The chemical factors may be agents such as anti-red cell antibodies, complement-induced membrane injury, disruption of the membrane integrity due to bacteria, toxin or chemical agents. Some of the chemical agents that have been reported to induce frank hemolysis in human red blood cells include aniline, apiol, arsine gas, chlorates, copper, formaldehyde, lead, mephenesin, nitrobenzene, resorince and spider and snake venoms. Seeman (1972) summarized the available data and suggested that chemicals such as anesthetics and tranquilizers increase the volume of red cells, and the surface area/volume ratio. There is also a 2% to 3% increase in membrane area. The mechanism of the interaction of anionic and cationic compounds with the red cell membrane is not completely understood. Anionic compounds cause externalization of the membrane by intercalating into the lipids in the external half of the bilayer, expand this half relative to the inner cytoplasmic half, and thereby induce discocyte-echinocyte transformation. On the other hand cationic amphiphiles intercalate in the inner bilayer leaflet and induce internalization of the membrane and discocyte-stomatocyte transformation (Lessin et al., 1983; Lourien and Anderson, 1982). Mohandas and Feo (1975) employed the anionic and cationic derivative of phenothiazine to demonstrate that the cellular concentration of the compounds increases linearly with solution concentration until there is hemolysis. Furthermore, significant differences were noted between the prelytic concentrations of anionic and cationic derivatives phenothiazine. Thus 160 million molecules of the cationic compound per red cell was necessary to induce the prelytic sphero-stomatocyte II stage, while about seven times as many molecules per cell were necessary to induce the prelytic sphero-echinocyte II stage. Complete hemolysis was noted at a cellular concentration of 5umoles/ml RBC with the cationic derivatives and 30umoles/ml RBC with the anionic derivatives. These differences suggest that the physico-chemical state of a molecule at physiologic intracellular pH would strongly influence its hemolytic properties. Similar analytic studies are needed to explain the hemolytic properties of EGBE and its metabolites. Figure 2 summarizes the shape changes of the discocyte induced by several important hemolytic mechanisms (Lessin and Lichtman, 1980).

A variety of compounds that produce active oxygen species, O_2^- as an intermediate in the reduction of molecular oxygen, induce structural and functional changes in erythrocyte membranes. Oxidative injury of the red cell membranes by ionic oxidants such as periodate has long been known to involve cleavage of C-C bonds between neighboring OH-groups in the sugar residues of glycoproteins (Furthmayr, 1977). Furthermore, periodate and iodate cause a time and concentration-dependent increase in erythrocyte membrane permeability for hydrophilic molecules and ions (Heller et al., 1984). Membrane damage in red cells in the presence of the radical-form of the oxidant, t-butyl-hydroperoxide is also well established. This altered permeability of the cell membrane is due to the formation of aqueous pores ($\sim$0.45 to 0.60nm). The leaks can be suppressed by various antioxidants (Deuticke et al. 1987). We have recently reported that tellurite (K_2TeO_3) alters red cell membrane and induces irreversible volume expansion, membrane vesiculation, increase the hydration state of the cells, and a decrease the mean cellular hemoglobin concentration (Kurantsin-Mills et al., 1988). We have also shown that there is increased specific membrane permeability to potassium ions several minutes prior to the volume change (Klug and Kurantsin-Mills, 1987).

II. STATEMENT OF THE PROBLEM AND OBJECTIVES OF THE STUDY

In the preceding discussion, we have summarized the significant species differences that have been reported for susceptibility to EGBE-related hemolysis. It is evident that frank hemolysis has been the major criterion for investigating the hematologic toxicity of EGBE and other glycol ethers. In order to gain significant insight into the EGBE-related hemolysis, it would be instructive to follow the process systematically as a function of EGBE concentration, time, and cellular responses prior to hemolysis. It would also be useful to measure those parameters that are related to the structural and functional integrity of the red cell. The results of the studies described in the following pages, form the basis of an initial attempt to establish an experimental approach to investigate the biophysical and biochemical mechanisms underlying EGBE-related hemolysis in the rat.

The original specific aims of this project were as follows:

1) To determine the effect of ethylene glycol monobutyl ether (EGBE) on the morphology of erythrocytes upon in vivo exposure in the rat to varying concentrations of EGBE in aqueous solutions for varying periods of time and the examination of the erythrocytes by scanning electron microscopy (SEM). Our hypothesis is that susceptible cells will display morphologic alterations as a function of time and concentration of EGBE.

2) To ascertain the effects of EGBE on the density distribution and thus, hydration state of red cells reflecting membrane alterations that result in net water and/or ion flux across the membrane. Our hypothesis is that such an ionic and water flux will result in alteration in surface area to volume ratio of the erythrocytes. The phthalate ester density distribution, an analytical technique, will be applied to determine the density profile of the cells.

3) To determine the effects of varying concentrations of EGBE on erythrocyte metabolism as assayed by the ability to generate adenosine triphosphate (ATP), a product of the glycolytic pathway. Our hypothesis is that changes in red cell ATP concentration may contribute to the EGBE-induced hemolysis.

4) To measure the effects of varying concentration of EGBE on the concentration of malonyldialdehyde (MDA) in erythrocyte membranes. Our hypothesis is that dose-related generation of oxidant radicals and red cell membrane injury by such oxidant radicals can contribute to the observed hemolysis.

5) To determine the effects of EGBE on red cell deformability utilizing a pressure-flow filtration technique. Our hypothesis is that the observed EGBE-induced hemolysis in the various species may be related to mechanical damage resulting from the shear stresses encountered in the microcirculation due to in surface area:volume ratio of the cells.

III. MATERIALS

Test Material: The test material, ethylene glycol monobutyl ether (EGBE), was supplied by Drs Kirk Dietz and A. T. Talcott of Dow Chemical Co., U.S.A., Midland, Michigan. The product, Dowanol(R) EB, ethylene glycol n-butyl ether (product code: 22366, CAS# 000111-76-2) was 99% pure. The physical and hazard data obtained from the analytical chemistry are summarized below.

PHYSICAL DATA
Boiling Point: 340°F
Vapour Pressure: 0.88mmHg @25°C
Vapour Density: (Air=1) 4.10
Sol. in water: Infinitely.
Sp Gravity: 0.897 @ 25/25°C
Appearance: Water white liquid
Odor: Ether-like odor

FIRE & EXPLOSION HAZARD DATA
Flash Point: 150°F
Method Used: TCC
Flammable Limits:
LFL: 1.1% Vol.
UFL: 10.6% Vol.
Extinguishing Media:
Alcohol foam, CO_2,
dry chemical.
Auto ignition temperature:is 471°F (244°C)

ANIMALS

The animals, male Sprague-Dawley rats (145-350 gm), aged 36-75 days were used in these studies. Animals were obtained from Charles Rivers Laboratories (Wilmington, MA) through the Animal Research Facility, The George Washington University Medical Center. The animals were individually identified with appropriate markings on their backs, and randomly assigned to control (non-test material exposed) or EGBE-exposed groups. They were allowed to acclimate to the laboratory conditions for several days prior to initiating the administration of EGBE. All animals were allowed food (Purina Certified Laboratory Chow, Ralston Purina, St. Louis, MO) and water ad libitum. Temperature and relative humidity in the animal holding facilities were maintained at approximately 23.5 $\pm$ 0.5°C and 60% respectively and the light cycle was regulated at 12 hr light, 12 hr dark. The animals were healthy throughout the study. There was no indication of any sickness of any animal during the study. The experimental animals were administered with EGBE dissolved in water at doses of 0, 50, 100, 250, 500 mg/kg by gavage. Blood samples were obtained at 0.5 hrs, 2hrs, and 4hrs for the analysis of the various parameters described in the following pages. Prior to the ingestion of EGBE, blood was sampled from the orbital sinus or by cardiac puncture of the rats with the use of anesthetics (Pentobarbital Sodium, USP, 40mg/kg), and analyzed for the parameters indicated under the specific aims.

Administration of Ethylene Glycol Monobutyl Ether to Rats.

Following the method of Ghanayem et al. (1987), the rats were administered with ethylene glycol monobutyl ether (EGBE) by gavage using a stainless steel feeding needle attached to 5 ml syringe. The EGBE, dissolved in water, was administered at a dose of 0mg/kg (control) to 500mg/kg. The volume administered per animal was maintained at 5 ml/kg. The animals tolerated the procedure well without any signs of gross immediate or delayed reactions. Five animals died after administration of 500 mg/kg EGBE, and

before the completion of 2 hr exposure to the compound for unexplained reasons. These animals appeared well, with no indication of ill health before the study. No post-mortem examination was done.

IV. EXPERIMENTS

Specific Aim 1: Effect of EGBE on Erythrocyte Morphology and Indices

Background and Rationale: The normal discocytic morphology of the red cell provides an optimal surface area (SA) to volume (V) relationship that facilitates its deformation in the microcirculation. The deformation of the red cell is determined by (a) the intracellular viscosity, which is a function of the mean corpuscular hemoglobin concentration; (b) membrane material properties including elasticity and viscosity; (c) cellular geometry (surface area to volume ratio, and morphology); and (d) the shear stresses acting on the cell surface. In the capillary of the intercordal splenic microcirculation, erythrocytes deform their shape tremendously to traverse vessels or pores with luminal diameters of about 3um (Chien et al., 1984; Kurantsin-Mills, 1988). If the deformability of the red cell is decreased, it loses its ability to traverse these small vessels, and premature destruction may occur. Furthermore, if hemolysis does occur, it will be reflected in changes in the red cell indices, and plasma and urine hemoglobin concentrations. EGBE-associated hemolysis may be preceded by alterations in SA/V ratio.

Materials and Methods

Red Cell Morphology: Red cell morphology was examined by scanning electron microscopy. The cells were fixed in 2% glutaraldehyde in phosphate buffered saline (PBS), pH 7.4, 295-300 mOsm/kg according to the method of Bessis and Weed (1972) and previously described by us (Kurantsin-Mills and Lessin, 1982). The cells were post-fixed in osmium tetroxide in PBS and dehydrated through graded ethanol (50% to 100%). The dehydrated cells were then infused with propylene oxide and air-dried on microscope studs. The cells were then coated with gold-palladium and examined in the scanning electron microscope (JSM 35U or ISI 100B).

Red Cell Indices: Red blood cell (RBC) volume and hemoglobin concentration distributions were measured by means of the Technicon H*1, flow cytometer, a technology that measures these cell indices by a laser light-scattering technique (Fig 3). The theoretical basis for these measurements as well as the optical and signal processing systems for the collection of data has been described in detail by Tycko et al. (1985) and Mohandas et al. (1986). The measured values of hemoglobin concentration for each red cell were utilized to construct a histogram of cell hemoglobin concentration. Hemoglobin distribution width (HDW) was derived as the standard deviation of the hemoglobin concentration histogram. For the analysis, 2ul blood was cytochemically prepared using a single buffered reagent (anionic surfactant and glutaraldehyde, pH 7.3). The result is isovolumetric spheres of red cells which flowed through the flow cytometer. Cell sizing was accomplished by differential light scattering.

Plasma and Urine Hemoglobin Concentrations: Absorption spectroscopy of hemoglobin has shown that the protein absorbs light strongly in the ultraviolet range. The band, 190-200 nm is associated with the peptide bond and 260-280 nm with the aromatic groups of tryptophan, tyrosine and phenylalanine. In addition, the heme moiety has characteristic spectra properties. Metal porphyrins have absorption spectra in specific wavelength bands of the electromagnetic spectrum including (a) the near ultraviolet (Soret band), 400-420 nm, with an extinction coefficient (mM) of 100 and (b) the visible bands longer wavelength, 650 nm and shorter wavelength, 550 nm with an extinction coefficient (mM) of 10. Plasma and urine hemoglobin concentrations were determined by use of these spectra properties of hemoglobin (Weissbluth, 1974; Beutler, 1984). Hemoglobin concentrations were measured by adding the standard hemolysate (0.3125 to 20 gm/dl) to 250 mg/l potassium ferricyanide and potassium cyanide (Fisher Diagnosis, Orangeburg, NY) reagent to convert the pigment to the stable cyanmethemoglobin, and reading the absorbance at 415nm and 540nm. The plasma and urine samples were similarly treated for estimation of hemoglobin concentration using the standard curves.

STATISTICAL METHODS

Data are presented as mean $\pm$ SD (SE), since all the variables analyzed are continuous in nature. Differences between group means, either the over all data or at each time of evaluation have been compared using analysis of variance (ANOVA) technique. Pairwise comparisons have been performed using protected Fisher's Least Significant Difference (LSD) procedure. p-values are reported as a two-tailed test at significance level of less than 0.05. Means with different letters are significantly different from each other at $p<0.05$. The number of rats used in each experiment is shown in the respective tables.

RESULTS

Red Cell Ultrastructural Morphology: Rats administered EGBE showed varying degrees of changes in the discocytic morphology of the RBCs, with subtle to definite formation of spherocytes. Detailed morphological analyses using scanning electron microscopy clearly demonstrated that the cells of the EGBE-treated rats had acquired spherocytic morphology (Figure 4), an indication of alteration in the surface area to volume ratio.

Peripheral Levels of Erythrocytes and their Indices: Measurements of red cell volume and hemoglobin concentration as well as their frequency distributions by the laser light-scattering technique confirmed the morphological observations. Figure 5 illustrates a typical cytogram output from the data processing component of the H*1 System showing changes in the mean cell volume (MCV) and the mean hemoglobin concentration (MCHC) frequency distribution for a control 145 gm rat (#23) and a 295 gm rat that received 500mg/kg EGBE (#32). The MCV frequency distribution curve broadened significantly. The mean cell volume increased from 53.8 fL to 86.9 fL. The frequency distribution of MCHC in contrast narrowed, and the mean value decreased from 35.2 gm/dl to 25.1gm/dl. Similar data as illustrated in Figure 5 were obtained for all animals studied, and the MCV, MCHC, RDW, and HDW values, as well as the other indices showed similar trends depending on the dose of EGBE administered and time of

exposure prior to the sampling of blood. As shown in Figures 6 and 7 the red cell MCV increased steadily whereas the MCHC decreased as a function of the dose of ingested EGBE, suggesting an increase in cell water content.

Table 2, presents the statistical data from all the hematologic parameters studied ignoring time and comparing the mean values of the control group with the EGBE-treated rats. With the exception of MCH, group comparisons in all the other parameters attained statistically significant levels at $p<0.001$. The mean values of MCV increased with increasing dose of ingested EGBE. The EGBE-treated animals had significantly higher MCV than the control group. The dose-response relationship noted for MCV was most apparent for 500 mg dose group which showed a significantly higher mean MCV values among the EGBE-treated rats as compared with the control group. Erythrocytes from the control animals had higher MCHC values than all EGBE-treated animals, with higher doses of the glycol ether resulting in lower MCHC values. Pairwise comparisons among group mean values in hematocrit, hemoglobin, red blood cell number, red cell density width, and hemoglobin density width, showed other significant group differences as presented by the last column of Table 2, but there was not a clear dose-response relationship as was observed for MCV and MCHC.

Using a 2-way ANOVA, with time and dose as factors, hematologic indices showing significant group mean differences are presented in Table 3. Evaluations performed at 0.5 hr and 2 hrs, consistently showed significant differences between 50 mg/kg and 500 mg/kg dose group in the following parameters: MCV, MCHC, HCT, RDW, HDW, HGB, and RBC. The middle doses seem to have comparable values during these times. During the half hour evaluation, the 500 mg/kg dose had a significantly higher mean MCV value 73.8 fL as compare to 61.0 fL for 50 mg/kg; lower MCHC value of 27.9 g\dl as compare to 33.6 g\dl for 50 mg/kg; higher HCT value of 44.4% as compare to 34.1% for 50 mg/kg; and higher HDW value of 2.23 g/dl as compare to 2.06 g\ld for placebo. The results of the 2 hr measurements were similar to the half hour data. The circulating RBC number was lower (4.98 E 6/uL) for the 500 mg/kg dose compared to 6.39 E 6/uL for the 50mg/kg dose. The trends at the 4 hr evaluation were very similar to those observed previously at the earlier time intervals.

Plasma and Urine Hemoglobin Concentrations

Absorption spectroscopy of hemoglobin at wavelengths of 415nm and 540nm demonstrated the presence of hemoglobin in the plasma and urine (Figure 8). Accurate estimates of hemoglobin concentration in plasma and urine were calculated from standard curves generated using hemoglobin solutions of known concentrations. Analysis of the plasma and urine samples of control rats which had not been exposed to EGBE revealed the presence of negligible concentrations of hemoglobin. Using these absorption properties of hemoglobin the presence of excess hemoglobin in the plasma and urine of rats two hours after the rats were administered EGBE was demonstrated. Furthermore, dose dependency was demonstrated two hours after the EGBE administration; increases of plasma and urine hemoglobin levels were detected with increasing dose (Table 4).

As evident from Table 4, the coefficient of variation of the estimated mean values are high, indicating the wide variation of plasma and urine hemoglobin values for the individual rats. A closer inspection of Table 4 also reveals that there is no linear trend between the ingested dose of EGBE and plasma hemoglobin concentration. However, despite the high coefficient of variation, the urine hemoglobin levels as assessed by its

absorption at 415 nm showed a definite linear increase from 50 mg/kg to 250 mg/kg within 2 hours after administration of EGBE. The urine hemoglobin measured at 540 nm did not show such a linear relationship to the ingested dose of EGBE. The difference is due to the difference in the sensitivity of hemoglobin absorption at the two wavelengths. These plasma hemoglobin measurements were done on samples that had been separated from the red blood cells by centrifugation immediately (within 10 minutes) after obtaining the blood. It is possible that if the hepatic metabolites of EGBE are stable in plasma, and the blood from a EGBE-treated rat is not centrifuged immediately to separate the cells and the plasma, the hemolytic process will continue on the bench and present artifactual results. Such unintended "bench hemolysis" can be incorrectly interpreted as an in vivo event*. The plasma hemoglobin concentration estimated by its absorbance at 415nm and 540nm were less than 5% and 1% respectively of the proportion of the total blood hemoglobin. The expression of the plasma and urine hemoglobin concentration as a function of the total blood hemoglobin concentration assumed that the total blood hemoglobin is far greater than that measured in both plasma and urine. Therefore the error entailed in the estimates is negligible, and percentages probably reflect the accurate level of hemolysis in the Sprague-Dawley rat under the condition of these studies. These observations contrast significantly with the report that the Fisher F344 rat exhibits high hemolysis, indeed as high as 40% to 50% total blood hemoglobin, when exposed to 500mg/kg EGBE (Ghanayem et al., 1987). A recent observation made in our laboratory has confirmed the suggestion that when plasma of EGBE-treated rats (50-500 mg/kg) is not separated from the red blood cells immediately (within 15 min), the cells continue to lyse at 25°C. Blood from control rats did not hemolyze under the same conditions. This indicates that the EGBE metabolites are stable in plasma at room temperature, and also confirm our suggestion that artifactual "bench hemolysis" can be misinterpreted as an in vivo event.

Specific Aim 2: Effect of EGBE on Erythrocyte Density Distribution

Background and Rationale: The hydration state of the red cell is an important determinant of its volume, the electrochemical distribution of ions such as Na^+, K^+ and Cl^-, the surface area to volume (SA/V) ratio, and hence its deformability and survival in the microcirculation. Oxidants are known to alter the red cell membrane integrity, resulting in an increased cell volume, until the critical hemolytic volume is attained and the cells lyse (Kurantsin-Mills et al., 1988). The rate of swelling has been related to the difference between the internal (p) and external (P) osmotic pressures or water activity. Therefore, the rate of swelling, $dv/dt = KA(p-P)$, where A is the surface area and K is a measure of water permeability (Ponder, 1948). An increase in the volume of the red cell is accompanied by a decrease in its density. Therefore, a measure of the density profile of the cell population would indicate changes in the density of the entire population or a fraction of them. The application of the nonaqueous phthalate ester differential density flotation system provides a simple, sensitive, and analytical approach for the correlation of the cell hydration, the cell internal viscosity and the cell geometry to the density profile (Kurantsin-Mills et al., 1987). Thereafter the density profiles of the red cells in control and EGBE-treated rats were evaluated to determine if the hemolytic process is selective for subpopulations of the cells.

Materials and Methods

The density distribution profile of the red cell was determined using the phthalate ester density technique. The details of the technique have been described elsewhere (Kurantsin-Mills et al., 1987). Briefly, the density distribution profiles of the various cell suspensions was analyzed in phthalate esters prepared from twenty mixtures of di-n-butyl phthalate (specific gravity, 1.042) and dimethyl phthalate (specific gravity, 1.189) (Fisher Scientific) with final specific gravity of the mixtures between 1.062 and 1.138 in increments of 0.004 (Weems and Lessin, 1984). Duplicates 60ul samples of the blood preparations were pipetted into microcapillary tubes (75 mm long, 1.15 mm internal diameter) containing 10 ul of each of the phthalate ester mixture. The end of the microcapillary tube in contact with the phthalate was sealed by flaming. The microcapillary tubes were then centrifuged at 13,460 g, for 15 min during which time the temperature equilibrated at 36.5°C (IEC MC Microhematocrit Centrifuge). The red cells distribution was analyzed by their differential floatation on the esters. The percentage of erythrocytes below the ester after the equilibrium centrifugation was related to the ester density using a computer program (PEDDS). The density profile curves generated from the data were analyzed for numerical indices that quantitatively described the properties of the cell population. These indices are the median density (D50), the density of the phthalate ester at which 50% of the cells are located after equilibrium centrifugation, and the 60% transitional density range over which the middle or transitional 60% of the cell population are located.

Results

Red Cell Density Distribution Profiles: Representative density distribution profiles of erythrocytes of control and EGBE-treated rats under ambient conditions and physiologic osmolality are shown in Figure 9. The median density (D50) is defined as the ester density at that point on the density profile dividing the cell population into two equal parts. Changes in the median density reflect an alteration in the density profile of the entire cell population. The 60% transitional density range (T60) is defined as the difference between the ester density values of the bottom 20% and the top 80% of the cells. The T60 is a numerical index of the homogeneity or heterogeneity of the cell population. The light cell fraction (LCF), $d < 1.096$ gm/ml and the dense cell fraction (DCF), $d > 1.106$ gm/ml describe the changes in the density profile following perturbation of the MCV and MCHC. Normal rat erythrocytes showed a narrow density distribution with the range from 1.0951 to 1.1088 gm/ml, a D50 value of 1.0994 ± 0.00453 gm/ml, and a (T60) value of 0.00623 ± 0.00183 gm/ml. The median density of rat RBCs treated with EGBE decreased as a function of the dose of the ingested compound (Table 5). The red cell density distribution profile of rats administered EGBE shifted to the left of the normal profile indicative of the increase in cell volume and decrease in cell hemoglobin concentration, perhaps as a result of increase in cell water content. There was a dose-dependent increase in the proportion of cells with median density less than the median density of the control cells (Figures 10). There was no specific effect on any subpopulations of cells according to the density profiles. The dose-response relationship between the ingested dose of EGBE and the alterations in the density properties of the circulating red cells (Figure 11), strongly suggests that all cells are equally affected by the

compound or by-products of its metabolism. These results add further support to the cellular alterations induced by EGBE and its metabolites as described above.

The use of a non-aqueous density separation system to measure the buoyant density of the red cells provided a sensitive and an analytical estimate of the effects of exposure to EGBE and its hepatic metabolites on the cells. The decrease in the median buoyant density of the cells of the treated animals is a reflection of the increase in cell volume and decrease in internal viscosity (vis-a-vis mean cellular hemoglobin concentration). At the critical hemolytic volume the cells would lyse. The potential of cell to lysis is therefore determined by the critical relationship between the surface area (SA) and the volume (V), expressed numerically as the SA/V ratio. The implications of these changes in SA/V ratio and buoyant density will become clearer in the subsequent studies under Specific Aim 5 on the rheological behavior of red cells of EGBE-treated rats.

Specific Aim 3: Analysis of Adenosine Triphosphate (ATP) Levels

Background and Rationale Glucose metabolism in the red cell via the glycolytic pathway results in the generation of high energy phosphate compounds, predominantly in the form of adenosine triphosphate (ATP). The ATP generated by this pathway is mainly utilized as a cofactor in the catalytic function of the ATPases that maintain the sodium-potassium and calcium pumps, which are essential for cation and cell volume homeostasis. The second pathway for glucose metabolism is the pentose phosphate pathway. In this pathway glucose 6-phosphate is oxidized at carbon-1 to yield CO_2. Concurrently $NADP^+$ is reduced to NADPH. NADPH is a primary substrate for the reduction of glutathione-containing disulfides in the red cell through the catalysis of glutathione reductase. This enzyme catalyzes the reduction of oxidized glutathione (GSSG) to the reduced form (GSH), and reduction of mixed disulfides of hemoglobin and GSH. An important function of GSH in the red cell is detoxification of low levels of hydrogen peroxide which may form spontaneously, or as a result of administration of a chemical. GSH also maintains the integrity of the cell by reducing oxidized sulfhydryl groups of hemoglobin, membrane proteins and enzymes, and preventing membrane lipid peroxidation by oxygen-derived reactive radicals, notably hydroxyl and superoxide anion) which may result in methemoglobin-dependent oxidative hemolysis (Benatti et al., 1982). A deficit of red cell ATP results in the disruption of cation and volume homeostasis, and to premature destruction of the erythrocyte in the microcirculatory systems of the spleen or liver. ATP concentration in the red cells of the control and treated rats were assayed to determine whether or not EGBE influences the energy state of these cells. The assay is based upon the enzyme catalyzed phosphorylation of glucose utilizing ATP, and subsequent oxidation of glucose-6-phosphate with the formation of NADPH and protons as described below.

Materials and Methods

The ATP content of the red cell preparations was determined by the method of Kornberg (1950) as modified by Lamprecht and Trautschold (1974). We have previously utilized this method to measure the ATP levels in diamide-treated human red cells (Kurantsin- Mills and Lessin, 1981). The assay can be expressed as follows:

(1) $\text{ATP} + \text{Glucose} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-PO}_4 + \text{ADP}$

(2) $\text{G-6-PO}_4 + \text{NADP}^+ \xrightarrow{\text{G6P-DH}} \text{6-PG-d-lactone} + \text{NADPH} + \text{H}^+$

For each mole of ATP, 1 mole of NADPH is formed. The reaction rate can be followed spectrophotometrically utilizing the extinction coefficient of NADPH at 340nm.

Fresh red cells were washed 3 times by suspension with cold PBS, pH 7.4 and resuspended to a low hematocrit (Hct). The cell suspensions were centrifuged at 1500xG for 10 min and the supernatant discarded. After the third wash, the cells were resuspended to about 50% Hct and exact Hct determined in triplicate. A perchlorate extract was then prepared using cold 0.6M perchloric acid. The extract was neutralized by titration with anhydrous potassium carbonate to pH 7.2, and the final extract stored at -20°C for 24 hours before the ATP assay was conducted.

Results

The concentration of adenosine triphosphate in the erythrocytes of normal healthy rats ranged from 0.771 mM/L cells to 1.164 mM/L cells. The average value for 24 rats was 0.940 $\pm$ 0.30 mM/L cell with an average coefficient of variation of 28.44% (Table 6). Our results compare very well with other reported values of red cell ATP levels in the rat (Bartlett 1976, Kreis et al.1981). Higher concentrations of RBC ATP were obtained in rats administered EGBE by gavage and the blood obtained two hours later for analysis. The doses of EGBE varied from 50 to 500mg/kg. Regardless of the volume of the cell, the mean red cell ATP levels were within one standard deviation of normal and not significantly different among the treated animals. Differences did not reach a statistically significant level ($p>0.05$).

Specific Aim 4: Effect of EGBE on Red Cell Lipid Auto-Oxidation

Background and Rationale

The peroxidation of unsaturated fatty acids of the red cell membrane has been suggested to contribute to hemolysis in several conditions. These include vitamin E-deficient states, thalassemia, and treatment with hyperbaric oxygen or oxidizing compounds. The mechanical behavior of the plasma membrane may also be affected by the peroxidation of endogenous membrane phospholipids. For example, tertiary butyl hydroperoxide induces peroxidation of unsaturated fatty acids in phospholipids of human red cells, and markedly increases the rigidity of the membrane and decreases cellular deformability (Corry et al., 1980). The peroxidation of unsaturated fatty acids in erythrocyte membranes leads to the formation of many aldehydes, ketones, and alcohols (Bidlack and Tappel, 1973; Porter, 1984). These compounds may alter the deformability characteristics of the red cell and contribute to hemolysis. Among the carbonyl compounds, malonyldialdehyde (MDA) has been studied most extensively, and has been widely used as an indicator of peroxidation of fatty acids. MDA concentration were measured to determine if EGBE affects the red cell peroxidation systems, thereby contributing to the hemolytic effect of this compound.

Material and Methods

The assay for MDA under oxidative stress is an index of the susceptibility of membrane lipids to auto-oxidation. The assay was carried out according to the method of Stocks et al (1972), with the modification that the final volume of hydrogen peroxide -cell suspension was 1.2 ml. EDTA was added to the cell suspensions at the thiobarbituric acid step in an attempt to inhibit possible Fe^{3+} enhancement color yield (Willis, 1964). MDA values are reported as nmole/gm hemoglobin. The preparation of red cell suspensions differed depending on the subsequent addition or omission of sodium azide.

For the experiment, the blood was centrifuged and the supernatant fluid and the buffy coat were removed. The red cells of control and EGBE-treated rats were washed in 50 times their volume of buffered saline with or without azide. After the second washing, a 2 ml suspension of 4% of these cells was pre-incubated at 37°C for 1 hr in a shaking water bath to inhibit catalase activity. A 0.5 ml of 40% trichloroacetic acid (TCA) was added to the final (4%) cell suspensions of control and EGBE-treated rats and then incubated at 22°C for 10 mins in a shaking water bath. The suspensions were centrifuged at 3500 rpm for 10 mins; the supernatant was carefully decanted without contaminating with the precipitate. One milliliter of 1% thiobarbituric acid (TBA) solution was added to the supernatant. An aliquot (4 ml) of the reaction mixture was placed in a boiling water bath for 15 mins, and then cooled immediately under cold water. The absorption spectra between 400 and 600 nm were obtained with a Beckman ACTA C II spectrophotometer using a reagent blank.

Results

Figure 13 illustrates the dose response relation between malonyldialdehyde (MDA) levels in the red cells and the administered dose of EGBE. The MDA concentration decreased to about 50% regardless of the administered dose. The decline in MDA concentration is suggestive of the arrest of autooxidative processes in the cells by EGBE or its metabolites. There was no evidence for an increase in membrane lipid peroxidation. Table 7 summarizes the statistics of the MDA data for the control and treated animals.

Specific Aim 5: Effect of EGBE on Whole Blood Viscosity Red Cell Deformability

Background and Rationale

Viscosity: The flow properties of a fluid is easily characterized by its viscosity. This variable can be derived from Newton's law which states that a correlation of the shear stress (dynes/cm^2) on the fluid with the rate of change of shear (shear rate, 1/sec) is a linear function, and that the slope of the line is the viscosity. Fluids that obey Newton's law are described as Newtonian and include water, physiological buffers, mercury and normal plasma. Unlike Newtonian fluids, the flow behavior of non-Newtonian fluids such as blood and other colloidal suspensions, may be both shear and time dependent. Such fluids exhibit a nonlinear shear stress versus shear rate plot. Therefore, blood does not flow until a critical shear stress, the yield stress, has been exceeded (Fig 14). Such fluids are considered to have a yield value or a gel strength and therefore described as pseudoplastic. Detailed analysis of the flow behavior of blood

reveals that it has a viscoelastic property; that is, there are viscous and elastic components to the process.

Blood is not a homogeneous fluid. Its flow behavior depends on the properties of the plasma proteins, the cells, and the interaction of plasma proteins with the red cells. Leukocytes and platelets normally exist in such low fractional volumes that they do not contribute significantly to whole blood viscosity. Under normal physiological conditions, the apparent viscosity of blood at a given temperature (e.g., 37°C) is a function of four main factors: (a) plasma viscosity, (b) cell concentration, (c) cell deformation, and cell aggregation (Chien, 1975, Kurantsin-Mills, 1988). The fluidity of the red cell is its most important property. It is dependent on (a) the fluidityof cell interior,; (b) membrane flexibility, and (c) the biconcave shape, determined by the excess surface area to volume ratio. In bulk flow, the membrane continuously rotates around the cell content, and the shear stresses are transmitted to the cell interior. Under these conditions the cell behaves like a fluid drop (Schmid-Schonbein et al., 1971). Because of these physiological factors and the effects of EGBE on the blood as described in the preceding pages, we considered it prudent to assess the whole blood viscosity of control and EGBE-treated rats.

Red Cell Deformability: The deformability of red cells (the change in shape of the cells in response to fluid shear) is now recognized as an important factor in the microcirculation. Several techniques are available to assess red cell deformability. Filtration of red cells as a measure of their deformability has been carried out under a variety of flow conditions. In some, the pressure required to filter a given volume of cells (of known concentration) is measured at a constant flow. In others, the volume filtered per unit time, under a constant pressure is determined. Some researchers measure the time required for the filtration of a known volume of the cell suspension (Barbenel, 1981; Adlercreutz et al., 1981; Schmalzer et al., 1983).

The reliance on a single index of the deformability of red cells has obscured the complexity of the filtration technique and the transient nature of a process. The process has various phases which are influenced by several factors including, entrance effects at the pores, contaminating leukocytes, plugging of the pores by the white cells or non-deformable red cells (Schmalzer et al., 1983). Skalak and associates have developed a method and a theoretical model for analyzing the pressure-time curve under constant flow rate during filtration (Schmalzer et al., 1983; Skalak, 1981 and Skalak et al., 1983). This model and pressure-time technique has been used to the study the effects of EGBE on red cell deformability.

Materials and Methods

Physiological Buffers

The physiological buffer for the in vitro filtration experiments was phosphate buffered saline (PBS) containing the following salts (in mM): NaCl, 145.5; KCl, 4.0; Na_2HPO_4, 1.65; KH_2PO_4, 0.16; D-glucose,11.1, buffered to pH 7.4 and containing 0.5% bovine serum albumin. The buffer was maintained at an osmolality 295-300 mOsm/kg measured by the freezing point technique with Microosmette Osmometer (Precision Systems Inc. Sudbury, MA). The pH of the buffer was determined by means of a pH-meter (Fisher Accumet pH-meter, Model 210).

The Preparation of Red Cells for Filtration

Fresh blood from the animals was treated with sodium heparin (14 u/ml) to prevent coagulation. The blood was then centrifuged at 1500 x G, 15 min, 4°C to separate the plasma and buffy coat. The red cells were washed with the phosphate buffered saline. The phosphate buffered saline was prefiltered through 0.2 um millipore membranes before use. The red cells were then be centrifuged through a column of Ficoll-Paque to remove the extra lymphocytes and granulocytes, washed 3 more times to remove the Ficoll-Paque and resuspended in PBS. The hematocrit was determined in triplicates by the microhematocrit method using a microcentrifuge (13,500g, 15 min). Ten milliliters of 0.2% cell suspensions was then pumped through 3u Nuclepore membranes using the Sage infusion pump and the initial pressure recorded within 1-5 seconds.

The Positive Pressure Filtration System and the Filtration of Cells

The essential features of the filtration (PPFS) apparatus has been previously described by us (Lessin et al., 1977). This apparatus was modified to permit measurement of the initial pressure within the first 1-5 seconds of flow of the suspending medium or the cell suspension. For the determination of red cell deformability, the pressure-time curve was recorded at a constant flow rate 30.0 ul/sec. A 10 ml plastic syringe was mounted on the Sage infusion pump (Model 355, Sage Instruments, Division of Orion Research Inc., Cambridge, MA) connected through the appropriate tubing to the Nuclepore chamber on which was mounted 13 mm polycarbonate filters (Nuclepore Corp. Pleasanton, CA) with nominal pore diameters 3 um. The buffer or the 0.2% cell suspension was pumped through the Nuclepore filter and the filtration pressure recorded as a function of time using a Validyne digital pressure transducer-manometer Unit (Model PS309, Validyne Engineering Corp, Northridge, CA) and a 10 inch recorder (Model L6012-1, Linseis Inc. Princeton JCT, NJ, 10in Recorder. The filtration of the buffer alone gives an initial pressure P_o. The filtration of the cell suspension gives a pressure, P_i followed by a gradual upward slope due to the plugging of pores by cells. Duplicate or triplicate preparations of the 0.2% cell suspensions were pumped through the apparatus for each group of animals. The numerical results of analysis were then averaged for each group.

Analysis of Filtration Data for the Deformability Index

By means of theoretical modeling, Skalak et al. (1983) have defined the relative resistance of an individual red cell in a pore of the Nuclepore membrane. The dimensionless parameter, B, is the ratio of the resistance in a pore bearing a cell to that in a pore filled with suspending medium only. The reciprocal of B, is an index of red cell deformability. The various dimensionless quantities defined by Skalak et al. (1983) relate pore radius, pore length, and red cell volume. These parameters can be related to the deformability index in the following equation, assuming the absence of leukocyte in the medium:

$$B\text{-}1 = [(P_i/P_o) - 1]\ V/h$$

where V is the volume ratio of erythrocyte to the pore, h is the fractional volume of red cells in the suspension, P_i is the initial pressure reading for red cell suspension pumped through the filter, and P_o is the value for the suspending medium alone.

Determination of viscosity

Rat whole blood was adjusted to a 40% hematocrit for the control and EGBE-treated experiments using autologous plasma, and used for the measurements of viscosity. Measurements were made on (a) 1 ml aliquot of autologous control plasma, (b) 1 ml aliquot of autologous plasma of EGBE-treated rat, (c) 1 ml aliquot of EGBE-treated red blood cells in autologous plasma at ambient conditions, (d) 1 ml aliquot normal red blood cells (control) in autologous plasma. Viscosity was measured in a Wells-Brookfield model LVT cone-plate microviscometer at a constant temperature of 37°C (Brookfield Engineering Labs., Inc., Stoughton, MA). Shear rate, calculated from rotation speed and the geometry of the cone and plate, was varied from 230 to 1.15 sec^{-1}. Shear stress was calculated from torque T in dyne centimeters acting on the cone of the viscometer (Wells et al., 1961; Reindorf et al.,1985). Apparent viscosity (n, hereafter referred to as viscosity) is defined as the ratio shear stress to shear rate in dyne-sec/cm^2 or poise. The result multiplied by 100 yields the unit centipoise.

Results

Viscosity of Plasma and Blood of Control and EGBE-treated Rats.

The relationship between viscosity and shear rate for the blood of control and EGBE-treated rats is illustrated in Figure 15. The suspensions exhibited a shear thinning behavior, ie, an inverse relationship between viscosity and shear rate. The viscosity of the plasma of the rats determined over a wide range of shear rates was relatively low. The plasma viscosity of control and EGBE-treated rats (500mg/kg) at a shear rate of 230 sec^{-1} averaged 1.45 $\pm$ 0.27cp and 2.50 $\pm$ 0.25cp respectively (Table 8). This difference in mean plasma viscosity was statistically significant at 95% confidence level. The treated animals showed an increase of 72% in plasma viscosity over the control animals. The viscosity of the whole blood of the control and the EGBE-treated rats was also shear dependent. As shown in Figure 15, the viscosity decreased with increasing shear rates and considerable differences in the viscometric behavior were observed among the two group of rats. At equal hematocrit of 40%, the mean blood viscosity of the rats that received 50mg/kg and 100mg/kg EGBE was 2 to 3-fold higher than that of the control rats. The mean viscosity of the rats that received 250mg/kg and 500mg/kg EGBE was lower than that at the other two doses, but higher than that of the control rats. This apparent lack of a dose-viscosity relation may be due to shear-induced hemolysis at the higher doses. The differences noted between the two group of rats are due to the alteration of the surface area to volume relationship of the red cells, apparent changes in plasma protein concentration as evidenced by the increased plasma viscosity of the EGBE-treated rats, and cell aggregation under the shear field of the viscometer. In order to elucidate the possible role of shear forces in the microcirculation on the hemolytic process, the blood from EGBE-treated (250-500mg/kg) rats was subjected shear forces in the viscometer and the concentration of hemoglobin released into the plasma compared to that of unsheared blood. Table 9 summarizes the results of these experiments. There was no difference in the plasma hemoglobin concentration of any of the treated animals whose blood was not sheared in the viscometer. The blood of the animals that received 500mg/kg EGBE had significantly increased plasma hemoglobin concentration compared to the control group and those that received 250mg/kg EGBE, at shearing forces 0.621 to 13.20 dynes/cm^2.

Filterability of Control and EGBE-Treated Rat Red Cells

The pore diameter of the filters used for the filtration of the red cells averaged 3.08 $\pm$ 0.27 and the pore density was (2.0 $\pm$ 0.1) x $10^6/cm^2$ in an effective filtration area of 0.78 cm^2. The pressure generated by 0.2% red cell suspension in PBS flowing through the polycarbonate filters at a constant flow rate of 30.0 ul/s displayed as a function of time for control and EGBE-treated erythrocytes exhibited the following characteristics. The pressure-time curves for the buffer and the control erythrocytes was biphasic, and the steady- state pressure values P_o and P_i were obtained in about 1-2 s. In contrast, the pressure-time curves of the suspension of EGBE-treated erythrocytes were multiphasic. Within the initial second, the pressure attained a quasi-steady value (P_i) that was higher than the P_i value for control red blood cells. Since the buffer and the cell suspension have been pumped through the Nuclepore filter pores at the same flow rate, the initial pressure values (P_o and P_i) have been normalized as the ratio P_i/P_o to reflect the rheological behavior of the suspensions (Kurantsin-Mills, 1979; Lessin et al., 1977; Schmalzer et al., 1983). Furthermore, the relative resistance of an individual red cell in a pore of the Nuclepore filter is defined by dimensionless parameter B, the ratio of resistance in a pore bearing a cell to that in a pore filled with the suspending buffer only. The reciprocal of B is an index of erythrocyte deformability. The initial P_i value for EGBE-treated cell suspensions reflect the pressure due to relatively deformable cells in transit, whereas the progressive rise in pressure after P_i is dependent on the plugging of the pores by the cells. The P_i/P_o value for control red blood cells for three rats ranged from 1.73 to 2.40 at a flow rate of 30.0 ul/s whereas that of four EGBE-treated rats ranged from 11.1 to 120 (Table 10). The difference between the P_i/P_o ratio for the control cells and those from EGBE-treated cells reflects the true deformability of the cells and was statistically significant ($P < 0.001$).

To determine the value of B for the control and the EGBE-treated red blood cells under these flow conditions, the average measured volumes of control red cells (55.5um') and EGBE-treated red cells (70.5 to 76.9um'), and the effective volume of the pore to 70.7um' for a pore diameter of 3 um were used. Thus, the deformability of the control cells ranged from 1.57 to 2.10 compared to that of EGBE-treated cells which varied from 11.59 to 127.60 (Table 10). The reduced flux of EGBE-treated cells through the pores is due to the change in the surface area to volume ratio and perhaps membrane viscoelastic properties.

V. DISCUSSION

The results of the studies summarized in the preceding pages demonstrate clearly that rats given EGBE by gavage show progressive alterations in the biophysical and biochemical characteristics of their red cells. The cell volume increases, the cell hemoglobin concentration decreases and the density distribution profile of the entire cell population shifts to lower density range. These effects of EGBE suggest that the red cell membrane has been altered by the oral administration of the glycol ether. This alteration in the cell membrane, the underlying mechanisms of which are currently unknown, results in the transmembrane flux of water and ions thereby increasing the cell volume, decreasing the surface area/volume ratio, and decreasing the cell hemoglobin concentration.

The scanning electron micrographs illustrate the changes observed in the red cell parameters for selected rats. The analytical techniques employed in these studies have provided relevant quantitative indices of the changes in the cells within the framework of the experimental design. The general trend observed among the control and EGBE-treated rats in terms of peripheral red cell levels, red cell indices and other parameters demonstrate significant differences between the control animals and the EGBE-treated rats. It is clear that there is a dose-dependent effect of the EGBE. Although the trends observed in the biophysical and biochemical properties of the cells exposed to EGBE and its metabolites are consistent, some of the measured variables indicated large variability. A time-dependent effect was also observed among the individual rats in the different experiments. This dose- and time-dependent effect of the compound is related to its biotransformation and the subsequent release of the metabolites into the circulation. Therefore, the pharmacokinetics of EGBE and its metabolites would determine their effects on various tissues *in vivo*.

Current biochemical evidence suggests that EGBE is metabolized in the liver to butoxyacetic acid (BAA), and glucuronide and sulfate conjugates of EBGE (Ghanayem et al., 1987). Using ^{14}C-labeled EGBE Ghanayem et al. (1987), identified butoxyacetic acid as the major urinary metabolite and the conjugated glucuronide and sulfate as minor metabolites. Based on these studies, the authors have proposed that EGBE is oxidized to the aldehyde, $CH_3(CH_2)_3OCH_2CHO$, an intermediate that is further oxidized to butoxyacetic acid, $CH_3(CH_2)_3OCH_2COOH$ in a reaction presumably catalyzed by the aldehyde dehydrogenase. These metabolic and biotransformation studies of glycol ethers have strongly suggested that the active hematotoxic compound is the carboxylic acid metabolite. This conclusion is also supported by studies by other investigators in which butoxyacetic acid was found as the major metabolite excreted by species used for the studies (Cheever et al., 1984; Bartnik et al., 1987). It would be desirable to investigate the effect of these metabolites in an appropriate in vitro experimental design in order to determine the specificity of these compounds on blood cells. Furthermore, the effects of these changes in the blood on its rheological properties and flow behavior in microcirculatory beds such as the liver and the spleen are unknown. These considerations will have to be investigated in an appropriate *in vitro*, *ex vivo* or *in vivo* model experimental systems.

The morphologic alterations induced by the ingestion of EGBE namely, sperocytes has been demonstrated by electron microscopy. We have applied the phthalate ester technique to analyze the effects of osmotic gradients across the red cell membrane on

their density distribution profiles. The median densities decreased as a function of decrease in the intracellular viscosity of the erythrocyte, as evidenced by the decrease in the MCHC. A correlation between D50 and the dose of ingested EGBE which altered the intracellular viscosity has been presented. The application of the phthalate ester density distribution method also provided important and significant insights into underlying cellular alterations that is reflected in the cell deformability. The hematological changes in EGBE-treated rats observed in the present study are not as pronounced for all the parameters, for example, plasma hemoglobin, hematocrit, RBC as reported by Ghanayem et al., 1987 for the Fisher 334 rats. Our data suggest possible differences in the susceptibility of different strains. Bartnik et al. (1987) employed cutaneous application of EGBE (260mg/kg and 500mg/kg) to assess *in vivo* hemolysis. The investigators noted relatively low hemolysis among the Wistar rats, although the data was not quantified. Direct intravenous administration of 62.5mg/kg EGBE resulted in no detectable hemolysis, whereas 75mg/kg increased the serum hemoglobin concentration by 25% above the mean value of the control in only 50% of the animals. This variability in the hemolytic effects of EGBE and/or its metabolites is consistent our observations in the present study. Ghanayem et al., (1987) reported in their studies with Fisher 344 rats 500mg/kg oral dose of EGBE increased plasma hemoglobin by about 5.0 g/dl within 4 hours in adult rats and 1.0g/dl in young rats. Furthermore, the animals recovered within 20 hours. Such a high plasma hemoglobin concentration in EGBE-treated F344 rats accounts for 45% to 50% of the total blood hemoglobin concentration, and should induce serious clinical complications in the animals. This is remarkably high increase plasma hemoglobin that not observed in the present studies using Spague-Dawley rats; and suggests significant methodological differences in the two studies. We have recently in our laboratory that when plasma of EGBE-treated rats (50-500 mg/kg) is not separated from the red blood cells immediately (within 15 min), the cells continue to lyse at 25°C. Blood from control rats did not hemolyze under the same conditions. This indicates that the EGBE metabolites are stable in plasma at room temperature, and also confirm our suggestion that artifactual "bench hemolysis" can be misinterpreted as an in vivo event.

The findings of this study also reveal that EGBE and/or its metabolites have profound effect on erythrocyte membrane structure that may result in rapid hemolysis. This raises the possibility that hemolysis is the result of a direct interaction between EGBE and/or its metabolites and the plasma membrane of the erythrocytes. The mechanism of interaction of EGBE or its metabolites with the erythrocyte membrane is unknown. As stated above anionic compounds cause externalization of the membrane by intercalating into the lipids in the external half of the bilayer, expand this half relative to the inner cytoplasmic half, and thereby induce discocyte-echinocyte transformation. On the other hand cationic amphiphiles intercalate in the inner bilayer leaflet and induce internalization of the membrane and discocyte-stomatocyte transformation (Lourien and Anderson, 1982). Analytical calculations for the inner and outer leaflets of the red cell membrane show significant differences in the volumes and areas two monolayers; an observation that supplements the biochemical data (Beck, 1978). At physiologic pH butoxyacetic acid may exit as an anion. Data presented by other investigators using radiolabeled EGBE (Bartnik et al., 1987; Ghanayem et al.,1988) show that BAA at low, non-hemolytic concentrations binds largely to the red cell membrane. However, the binding and distribution of BAA in membrane lipids and proteins is not known. The differences in the lipid and protein composition red cells from different species or strains

of the same species may also influence the binding of the glycol ether and its metabolites.

Chemical-induced hemolysis is currently thought to occur by either of two mechanisms. The first involves direct interaction of the chemical with the red cell membrane which results in changes in membrane structure, increased permeability to water and ions, osmotic swelling and hemolysis. In the second mechanism, the chemical penetrates the membrane into the cell interior where it interferes with cellular metabolism ultimately resulting in membrane damage and hemolysis. The drug-induced hemolysis in the second mechanism may be due to enzyme deficiencies, unstable hemoglobin.

Identification of the binding site on the erythrocyte membrane surface would help for a better understanding of the mechanism of action of butoxyacetic acid and the other metabolites of EGBE. There may be significant variation in the hemolytic effect of butoxyacetate on erythrocytes from different species or strains of the same species. The basis for the reported differences in the hemolytic tendency among species is not known but variation in the protein and lipid composition of the red cell membrane may account for such differences. On the basis of the results of this study and others (Carpenter et al., 1975; Ghanayem et al.,1987; Dodd at al., 1983), we suggest that EGBE and/or its metabolites interacts directly with the red cell membrane and induces permeability alterations. Since these compounds have hydrophobic segments, the interaction may be of a hydrophobic type, and would lead to an impairment of membrane function. Further studies are needed to elucidate these suggestions.

We had hypothesized that changes in the red cell energy levels (ATP,) may contribute to the disruption of the membrane in EGBE-treated rats. This proposition was based on the rationale that ATP is important in ion and water homeostasis, as well as glutathione metabolism. In the light of the coefficient of variations of the mean values of the ATP levels found in the red cells, we conclude that there was no statistically significant difference between the control rats and the EGBE-treated group. The effects of butoxyacetic acid (BAA), and glucuronide and sulfate conjugates of EBGE on red cell metabolism have not been studied. It would be instructive to investigate the effects of these compounds on substrate transport, red cell, metabolism, membrane permeability, and the mechanisms of the hemolytic process. The differences between species in some of these ATP-regulating processes can then be evaluated and compared.

The observation that MDA concentration in red cells decreased after oral administration of EGBE to rats is indicative of the arrest of autoxidative processes by EGBE or its metabolites. In the normal physiological state, the erythrocyte is resistant to oxidative damage because of a number of intrinsic protective antioxidant mechanisms. These protective mechanisms include catalase, and glutathione (GSH), among others (Porter, 1984). Although the red cells of freshly drawn blood contain no detectable lipid peroxide or other lipid autoxidation products, the cells can be induced to auto-oxidize by inhibition of some of the antioxidants. The formation of malonyldialdehyde (MDA) as a secondary breakdown product of fatty-acid peroxides under such conditions has been used as a measure of autoxidation (Stocks and Dormandy, 1971). Membrane lipid peroxidation is a common occurrence in certain types of hemolytic anemia, and chemical-induced membrane injury (Deuticke et al., 1987a and 1987b). Other studies have shown that accumulation of MDA in red cells can reduce the deformability of red cells. We suggest that butoxyacetic acid, a major by product of hepatic metabolism of EGBE may be acting as a free radical scavenger, and therefore an antiperoxidative molecule, thereby reducing the concentration of membrane MDA. This suggestion is supported by the

observation that other compounds with similar chemical structure (an aliphatic chain with a carboxylic acid at its end, R-COOH) have been shown to have antiperoxidative properties and protect ischemic tissue (Mak and Weglicki, 1988). If the suggestion that EGBE metabolites are free radical {such as superoxide, hydroxyl or lipid peroxide} scavengers is valid, it should be possible to detect the presence or absence of these labile intermediate molecules using direct detection by electron spin resonance [ESR] spectroscopy or spin trapping and ESR.

The observation that the treated animals showed an increase of 72% in plasma viscosity over the control animals is indicative of significant alterations in the plasma protein concentration. The viscosity of plasma in the normal rat is fairly constant in the normal state. This constancy implies a strict dynamic equilibrium between the synthesis and release of new plasma proteins into the circulation, and the continual catabolism of these proteins. Since plasma viscosity is primarily a function of plasma protein concentration, the 72% increase noted in the EGBE-treated rats strongly suggest that the glycol ether has induced significant changes in the plasma protein levels. The mechanism by which plasma proteins alter plasma viscosity is due to the hydrodynamic influence of the molecules, which is a function of the volume concentration of the protein, and the hydrodynamic variation in the effective molecular volume (Kurantsin-Mills, 1988). Since fibrinogen has the largest axial asymmetry, it has the greatest effect on plasma viscosity, although albumin and the globulins may contribute such an increases. It would therefore be instructive and useful to analyze the protein fractions of EGBE-treated rats in order to elucidate the specific changes in plasma proteins. It is possible that hepatic metabolism of EGBE is accompanied by alterations in the hepatic output of plasma proteins.

The increased plasma protein concentration, reflected in the increased plasma viscosity, and the decreased red cell deformability reflected in the altered filterability explain the increased whole blood viscosity observed among the EGBE-treated rats for 50mg/kg and 100mg/kg doses. There was an apparent lack of a dose-viscosity relation noted for the different groups of rats at 250mg/kg and 500mg/kg that may be due to shear-induced hemolysis at the higher doses. Since red cell aggregation is induced by the bridging of adjacent cells by fibrinogen, globulins, and other large molecules in the plasma (Chien, 1975, 1980), increased plasma protein concentration will cause increased cell aggregation that will be reflected in the whole blood viscosity. Under the low flow states encountered in the microcirculation, the aggregated red cells with altered membrane properties can lyse. The role of shear forces in the microcirculation on hemolysis in EGBE-treated rats was verified by subjecting the blood to shear forces in the viscometer, and the concentration of hemoglobin released into the plasma compared to that of unsheared blood. The plasma hemoglobin concentration of treated animals, whose blood was not sheared in the viscometer did not vary significantly. However, the animals that received 500mg/kg EGBE had significantly increased plasma hemoglobin concentration compared to the control group. This difference suggest that the normal shear stresses that disperses aggregated red cells in the microcirculation may have contributed to the observed hemolysis. Because erythrocytes deformability through the microcirculation is a function of SA/V ratio, intracellular viscosity and membrane viscoelastic properties, we believe that application of multiple techniques, including viscometric and filtration measurements, has provided some insight into the rheological behavior of the red cells of the EGBE-treated rats *in vivo*.

References

Barbenel JC: The measurement of red blood cell deformability. In: Lowe GDO, Barbenel JC and Forbes CD (eds), Clinical Aspects of Blood Viscosity and Cell Deformability. Springer-Verlag Berlin. pp. 37-45, 1981.

Bartnik FG, Reddy AK, Klecak G, Zimmermann V, Hostynek JJ and Kunstler K. Percutaneous absorption, metabolism and hemolytic activity of n-butoxyethanol. Fund. and Appl. Toxicol. 8:59-70, 1987.

Beck JS. Relations between membrane monolayers in some red cell shape transformations. J. Theor. Biol. 75:487-501, 1978.

Benatti U, Morelli A, Damiani G and De Flora A. A methemoglobin-dependent and plasma-stimulated experimental model of oxidative hemolysis. Biochem. and Biophys. Res. Comm. 106(4):1183-1190, 1982.

Bessis M, Weed RI. Preparation of red blood cells (RBC): A survey of various artifacts. Scanning Electron Microscopy (Part 11). Proc. of the workshop on Biological Specimen Preparation Techniques for Scanning Electron Microscopy; IIT Research Inst., Chicago, Ill. 5:289-296, 1972

Bidlack WR and Tappel AL. Fluorescent products of phospholipids during lipid peroxidation. Lipids. 8(4):203-207, 1973.

Carpenter CP, et al.: The toxicity of butyl-cellosolve solvent. A.M.A. Arch. of Indust. Health. 14:114-131, 1956.

Carpenter CP, Kinkead ER, Geary DL, Sullivan LJ and King JM. Petroleum Hydrocarbon Toxicity Studies: Methodology. Toxicol. and Applied Pharmacol. 32:246-262, 1975a.

Carpenter CP, Kinkead ER, Geary Jr DL, Sullivan LJ and King JM. Petroleum and Hydrocarbon Toxicity Studies: Animals and human responses to vapors of varnish makers' and painters' naphtha. Toxicol. and Applied Pharmacol. 32:263-281, 1975b.

Cheever KL, Plotnick HB, Richards DE and Weigel WW. Metabolism and excretion of 2-Ethoxyethanol in the adult male rat. Environ Health Persp. 57:241-248, 1984.

Chien S. Aggregation of red blood cells: An electrochemical and colloid chemical problem. In "Bioelctrochemistry: Ions, Surfaces, Membranes," Adv. Chem. Ser., Am Chem. Soc., Washington, D.C. (C) 1-32, 1980.

Chien S. Biophysical behavior of red cells in suspensions. In "The Red Blood Cell" (D. MacN. Surgenor, ed.) 2nd ed., Academic Press, New York. 2:1031-1133, 1975.

Corry WD, Meiselman HJ and Hochstein P. t-Butyl hydroperoxide-induced changes in the physicochemical properties of human erythrocytes. Biochimica et Biophysica

Deuticke, B, Heller, KB and Haest, CWM. Progressive oxidative membrane damage in erythrocytes after pulse treatment with t-butylhydroperoxide. Biochimica et Biophysica Acta, 899, 113-124, 1987a.

Deuticke, B, Lutkemeier, P and Sistemich, M. Uncoupling oxidative leak formation from lipid peroxidation in human eryhtrocyte membrane by antioxidants and desferrioxamine. Biochimica et Biophysica Acta, 899, 125-128, 1987b.

Dodd DE, Snellings WM, Maronpot RR and Ballantyne B. Ethylene glycol monobutyl ehter: acute, 9-day and 90-day vapor inhalation studies in fischer 344 rats. Toxicol and Applied Pharmacol 68:405-414, 1983.

Dugard PH, Walker M, Mawdsley SJ and Scott RC. Absorption of some glycol ethers through human skin in vitro. Environ Health Persp. 57:193-197, 1984.

Fischer TM, Haest SWM, Stohr M, Kamp D and Dentick B: Selective alteration of erythrocyte deformability by SH-reagents. Biochem. Biophys. Acta. 510:270-282, 1978.

Furthmayr H. Journal of Supramolecular Structure. 7: 121-134, 1977

Ghanayem BI, Burka LT, Sanders JM, Matthews HB. Metabolism and disposition of ethylene glycol monobutyl ether (2-Butoxyethanol) in rats. Drug Metabolism and Disposition. 15, 478-484, 1987

Ghanayem BI, Blair PC, Thompson MB, Maronpot RR and Matthews HB. Effect of age on the toxicity and metabolism of ethylene glycol monobutyl ether (2-Butoxyethanol) in rats. Toxicol and Applied Pharmacol 91:222-234, 1987.

Ghanayem BI, Burka LT and Matthews HB. Metabolic basis of ethylene glycol monobutyl ether (2-Butoxyethanol) toxicity: role of alcohol and aldehyde dehydrogenases. J. of Pharmacol and Exper. Therap. 242(1):222-231, 1987.

Ghanayem BI, Burka LT, Sanders JM and Matthews HB. Metabolism and disposition of ethylene glycol monobutyl ether (2-Butoxyethanol) in rats. Drug Metab. and Disp. 15(4)478-484, 1987.

Grant D, Sulsh S, Jones HB, Gangolli SD and Butler WH. Acute toxicity and recovery in the hemopoietic system of rats after treatment with ethylene glycol monomethyl and monobutyl ethers. Toxicol. and Appl. Pharmacol. 77:187-200, 1985.

Groeseneken D, Veulemans H and Masschelein R. Respiratory uptake and elimination of ethylene glycol monoethyl ether after experimental human exposure. Brit. J. of Indust. Med. 43:544-549, 1986.

Hanss M: Erythrocyte Filtrability Measurement by the initial flow rate method. Biorheology 20:199-211, 1983.

Jonsson A-K and Steen G. n-Butoxyacetic acid, a urinary metabolite from inhaled n-butoxyethanol (Butylcellosolve). Acta Pharmacol et Toxicol. 42:354-356, 1978.

Kornberg A. Reversible enzymatic synthesis of diphosphopyridine nucleotide and inorganic pyrophosphate. J. Biol. Chem. 182, 779-793, 1950.

Kurantsin-Mills J, and Lessin LS. Aggregation of intramembrane particles in erythrocyte membranes treated with diamide. Biochemica et Biophysica Acta, 641, 129-137. 1981

Kurantsin-Mills J, Lessin L. Rheological assessment of the antisickling properties of pyridoxal and pyridoxine. Blood Cells 8, 315-328, 1982

Kurantsin-Mills J and Lessin LS. Cellular and rheological factors contributing to sickle cell microvascular occlusion. Blood Cells. 12:249-270, 1986.

Kurantsin-Mills J, Jacobs HM, Lessin LS: Assessment of hydration state of sickle cells by phthalate ester density distribution. J. Lab Clin Med 109, 486-494, 1987.

Kurantsin-Mills J. Blood rheology and the microcirculation. In The Basic Science of Vascular Surgery. eds. Giordano JM, Trout HH III and DePalma RG. Futura Publishing Company Inc., Mount Kisco, NY., 1988.

Kurantsin-Mills J, Klug RK and Lessin LS. Irreversible erythrocyte volume expansion induced by tellurite. Brit. J. Haematol. 70:369-374, 1988.

Lamprecht W, Trautschold I. Adenosine-5-Triphosphate: Determination with hexokonase and glucose-6-phosphate dehydrogenase. In Methods of Enzymatic Analysis. Hans U. Bergmeyer ed. Vol. 4, 2101-2110. Weinheim, Verlag Chemie, New York, London Academic Press, 1974.

Lessin LS and Lichtman MA: In: The Science and Practice of Clinical Medicine, Hematology and Oncology. Lichtman MA (ed). Grune and Stratton Inc., New York, p. 50, 1980.

Lessin LS, et al.: Morphology of the erythron. In Williams W, et. al. (ed). Hematology, McGraw Hill, New York, pp. 257-259, 1983.

Lessin LS: Assessment of hematologic toxicity of ehtylene glycol monobutyl ether (EGBE): Technical report for the Chemical Manufacturer's Association. Washington, D.C. pp. 1-33, 1985.

Lovrien R and Anderson RA. Critical lipid-protein stoichiometries in erythrocyte membrane reactions governing protection and morphology switching. Biophys. J. 37:12-14, 1982.

Mak T and Weglicki WB. Protection by beta-blocking agents against free radical-mediated sarcolemmal lipid peroxidation. Circ. Res. 63(1): 62-266, 1988.

Mohandas N, and Feo C. A Quantitative Study of the red cell shape changes produced by anionic and cationic derivatives of phenoithiazine. Blood Cells 1: 375-384, 1975.

Mohandas N, Kim YR, Tycko DH, Orlik J, Wyatt J, Groner W. Accurate and independent measurement of volume and hemoglobin concentration of individual red cells by laser light scattering. Blood 68, 506-513, 1986

Mohandas N, Phillips Wm and Bessin M: Red blood cell deformability and hemolytic anemias. Seminars in Hematology 16:95-114 (2), 1979.

Porter NA. Chemistry of Lipid Peroxidation. Methods In Enzymology, 105:273-282, 1984.

Reindorf CA, Kurantsin-Mills J, Allotey JB and Castro O. Perflurocarbon compounds: effects on the rheological properties of sickle erythrocytes in vitro. Am. J. Hematol. 19:229-236, 1985.

Rowe VK and Wolf MA. Derivatives of glycols. In Clayton GD, Clayton FE, eds. Patty's Industrial Hygiene and Toxicology. Vol 2C, 3rd rev, John Wiley, New York, pages 3909-4052, 1982.

Schmalzer EA, Shalak R, Usami S, Vayo M and Chien S: Influence of red cell concentration of filtration of blood cell suspension. Biorheology. 20:29-40, 1983.

Schmid-Schonbein H, Wells RE and Goldstone J. Fluid drop-like behaviour of erythrocytes- disturbance in pathology and its quantification. Biorheology. 7:227-234, 1971.

Seeman P. The membrane actions of anesthetics and tranquilizers. Pharmacol. Reviews. 24(4):583-655, 1972.

Skalak R: Theoretical models of deformability of blood flow. Scand. J. Clin. Lab. Invest. 41, Suppl 156, 55-58, 1981.

Skalak R, Impelluso T, Schmalzer EA and Chien S: Theoretical modeling of filtration of blood cell suspension. Biorheology 20:41-56, 1983.

Sparer J, Welch LS, McManus K, and Cullen MR. Effects of Exposure to Ethylene Glycol Ethers on Shipyard Painters: I. Evaluation of Exposure. American J. Industrial Medicine 14: 497-507, 1988.

Stocks J, Offerman EL, Modell CB and Dormandy TL: The susceptibility to auto-oxidation of human red cell lipids in health and disease. British J. HAematol. 23:713-724, 1972.

Tycko DH, Metz MH, Epstein EA, Grinbaum A. Flow-cytometric light scattering measurement of red cell volume and hemoglobin concentration. J. Appl Optics, 24, 1355-1360, 1985

Tyl RW, et. al.: A teratologic evaluation of ethylene glycol monobutyl ether in Fischer 344 rats and New Zealand white rabbits following inhalation exposure. Union Carbide Corp. Bushy Run Research Center Project Report. 46-521, 1984.

Weissbluth M. Hemoglobin: cooperativity and electronic properties. Sringer-Verlag, Heidelberg, pp 125-141, 1974.

Wells RE, Denton R and Merrill EW. Measurement of viscosity of biologic fluids by cone plate viscometer. The J. Lab. and Clin. Med. 57(4):646-656, 1961.

Werner HW, et. al.: The acute toxicity of vapors of several monoalkyl ethers of ethylene glycol. J. Indust. Hyg. and Toxicol. 25:157-163, 1943a.

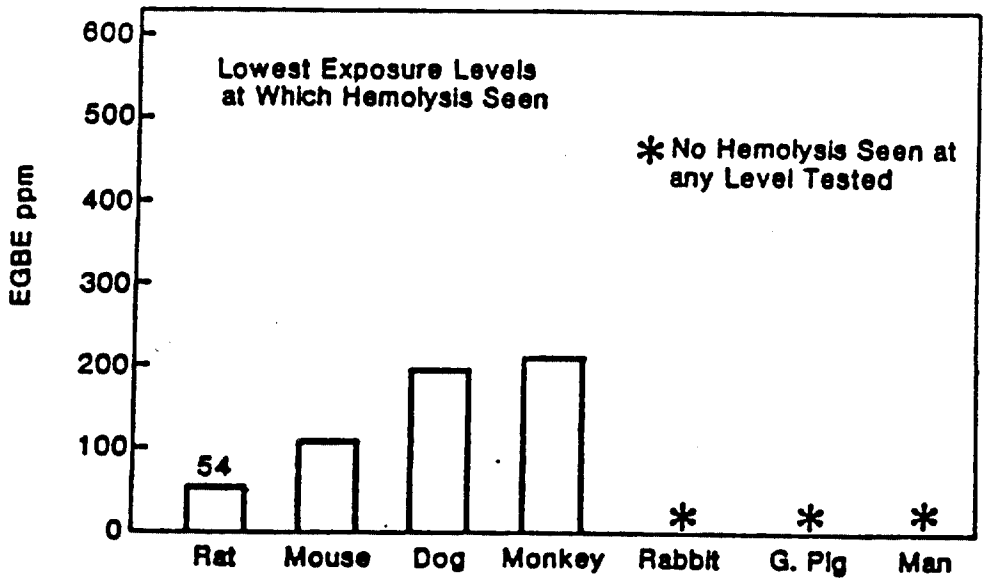
Werner HW, et. al.: Effects of repeated exposures of rats to vapors of monoalkyl ethylene glycol ethers. J. Indust. Hyg.and Toxicol. 25:374-379, 1943b.

Werner HW, et. al.: Effects of repeated exposures of dogs to monoalkyl ethylene glycol vapors. J. Indust. Hyg. and Toxicol. 25:409-414, 1943c.

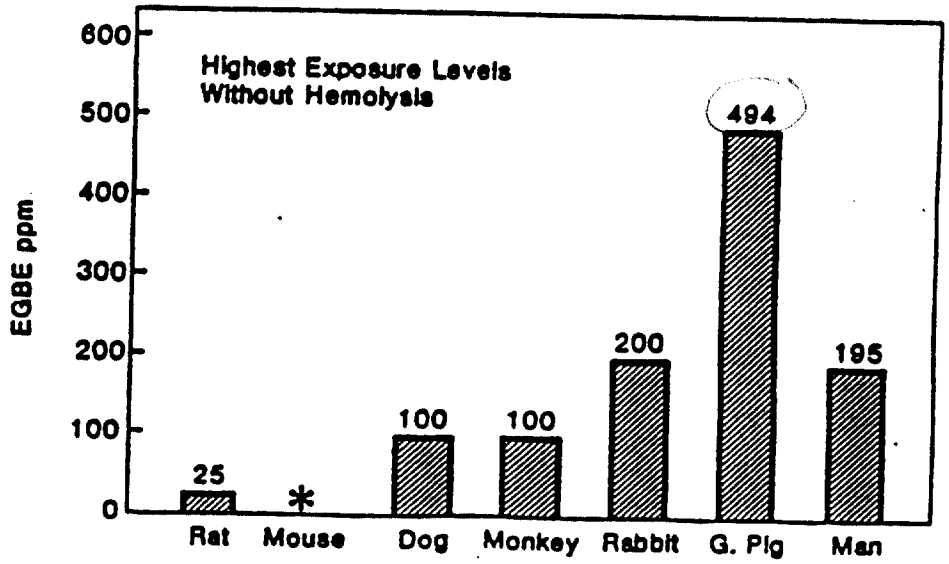
Willis ED: The effect of inorganic iron on thiobarbituric acid method for the determination of lipid peroxide. Biochem. Biophys. Acta. 84:475-477, 1964.

FIGURE 1

Hemolytic Thresholds for EGBE Vapor Exposures-Species Differences



Hemolytic Resistance to EGBE Vapor Exposures-Species Differences



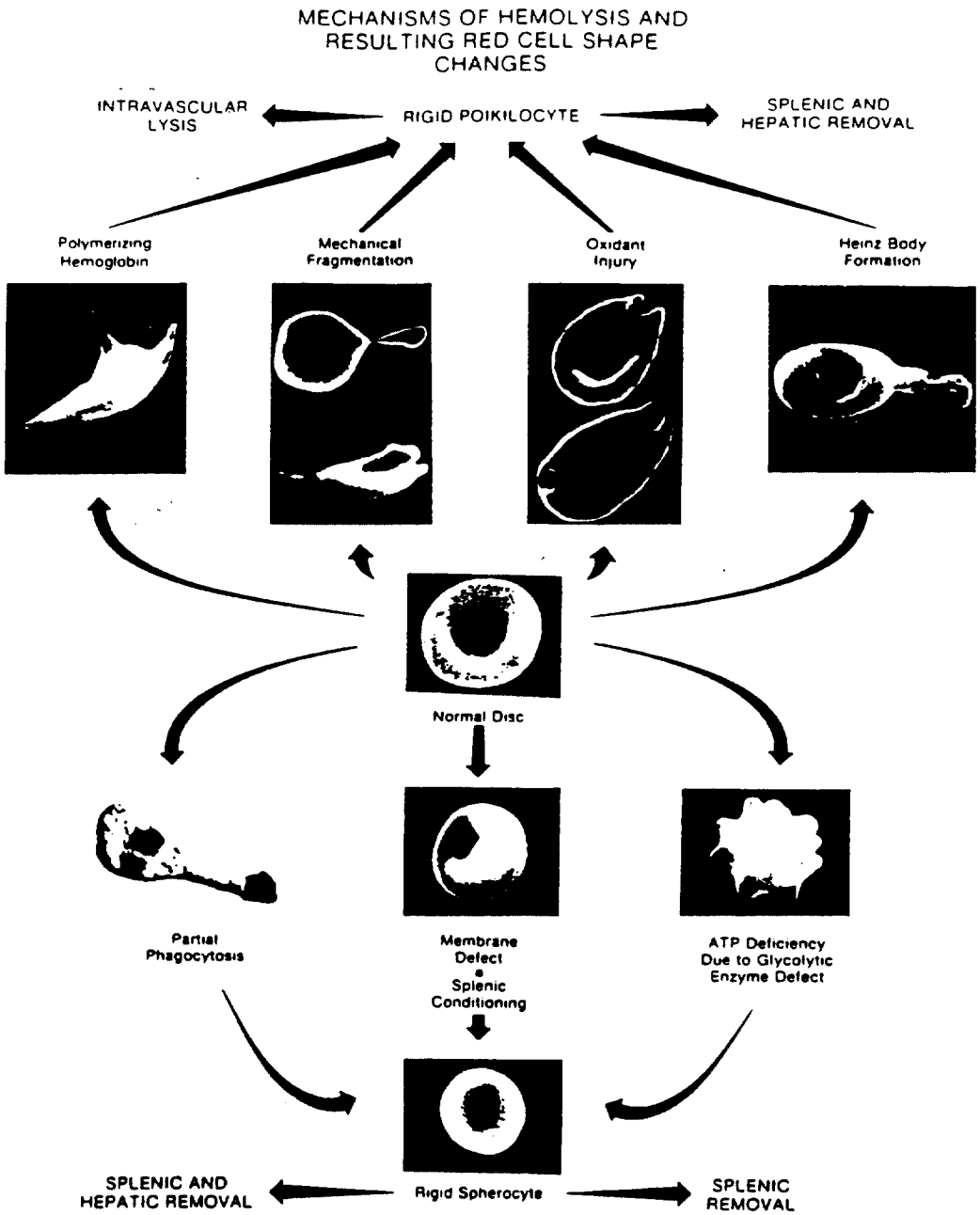


Figure 2 This schematic drawing depicts the alterations from the disk shape by several important hemolytic mechanisms. The red cells are shown using scanning electron microscopy. Polymerizing hemoglobins like sickle cell disease, fragmentation of red cells in the microcirculation or the heart, oxidant injury from G-6-PD deficiency, and Heinz body formation from thalassemic disorders are depicted as leading to the formation of rigid poikilocytes. These processes may lead to intravascular as well as to hepatic and splenic destruction of cells. Not shown is the intravascular hemolysis that occurs with complement lysis of cells.

Partial phagocytosis as occurs in the warm-antibody type of immune hemolytic disease, spherocytosis as a result of an inherited membrane disorder, and spheroechinocytosis from ATP deficiency are depicted as leading to spherocytes or closely related shapes. These processes rarely result in intravascular hemolysis. Exceptions to this are the profound spherocytic anemia of the exotoxinemia of clostridial septicemia and the profound warm-antibody type of autoimmune hemolytic disease.

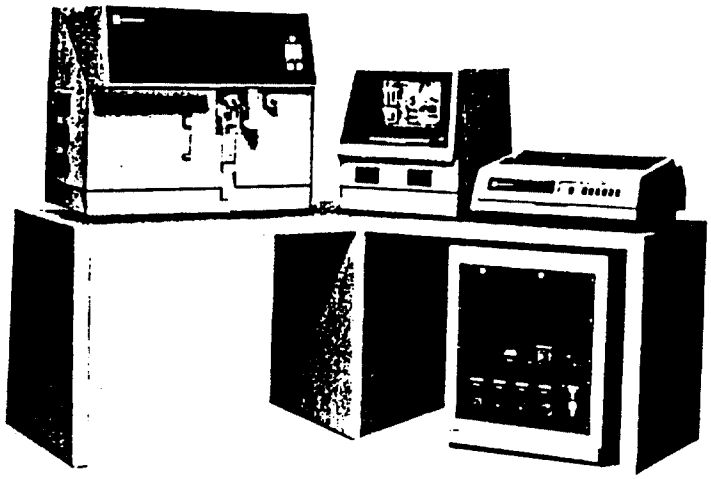


Figure 21: H·1 System

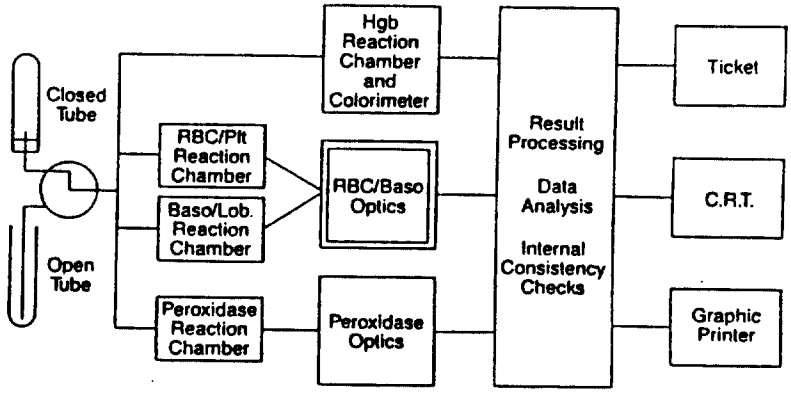


Fig 3: The TECHNICON H*1 SYSTEM: (A) Schematic illustration of the modular components of the instrument. (B) Diagram of the erythrocyte measurement system. A 20 x 150-um rectangular cross section of the flow cell is illuminated by the laser light source. Dilute erythrocyte suspension delivered by a peristaltic pump flows through the sheathed-stream flows at a linear velocity of approximately 1 m/s. Sample stream diameter is about 35 um. Measurements begin 50 seconds after mixing 10 ul a blood sample with the reaction buffer. Data is acquired for a period of 10 seconds during which 5,000 to 10,000 individual red cells are measured. A two dimensional (S_1, S_2) pulse height distribution is displayed on the oscilloscope for each sample from which volume and hemoglobin concentration histograms are generated.
(Adapted from Mohandas et al. Blood 68 (2), 506-513, 1986)

Figure 4: Red Cell ultrastructural morphology. Illustration of representative electron micrographs of red cells of control (top) and EGBE-treated [100mg/kg, 30min] rats. Note the spherocytic morphology of cells of the EGBE-treated rats.

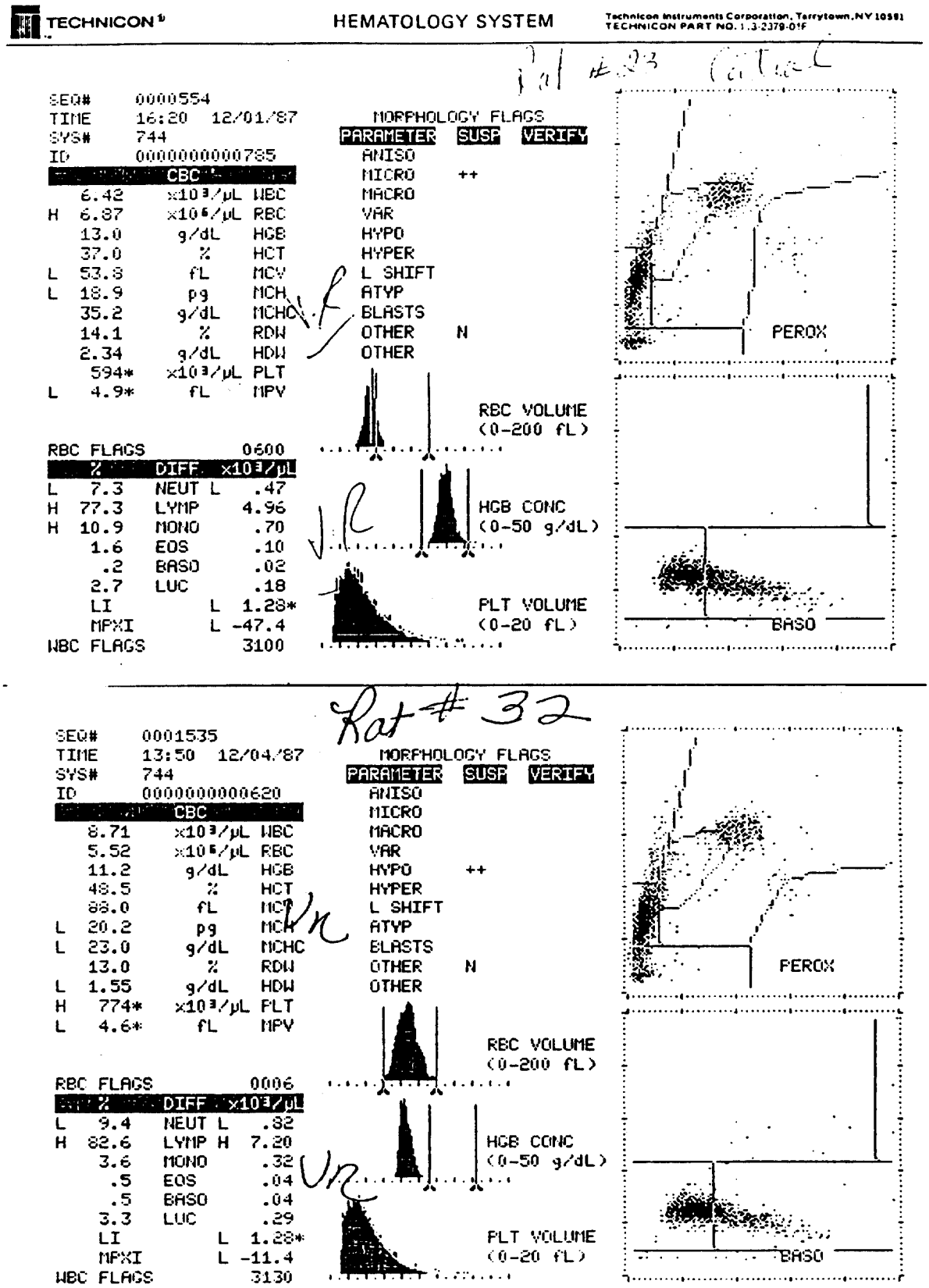


Figure 5: Representative flow cytometer output from data processing subunit of the H*1 System.

Figure 6: Dose-response relationship between the ingested dose of EGBE and the changes in the mean cell volume of the circulating red blood cells in the rat given EGBE by gavage for 0.5, 2, or 4 hours.

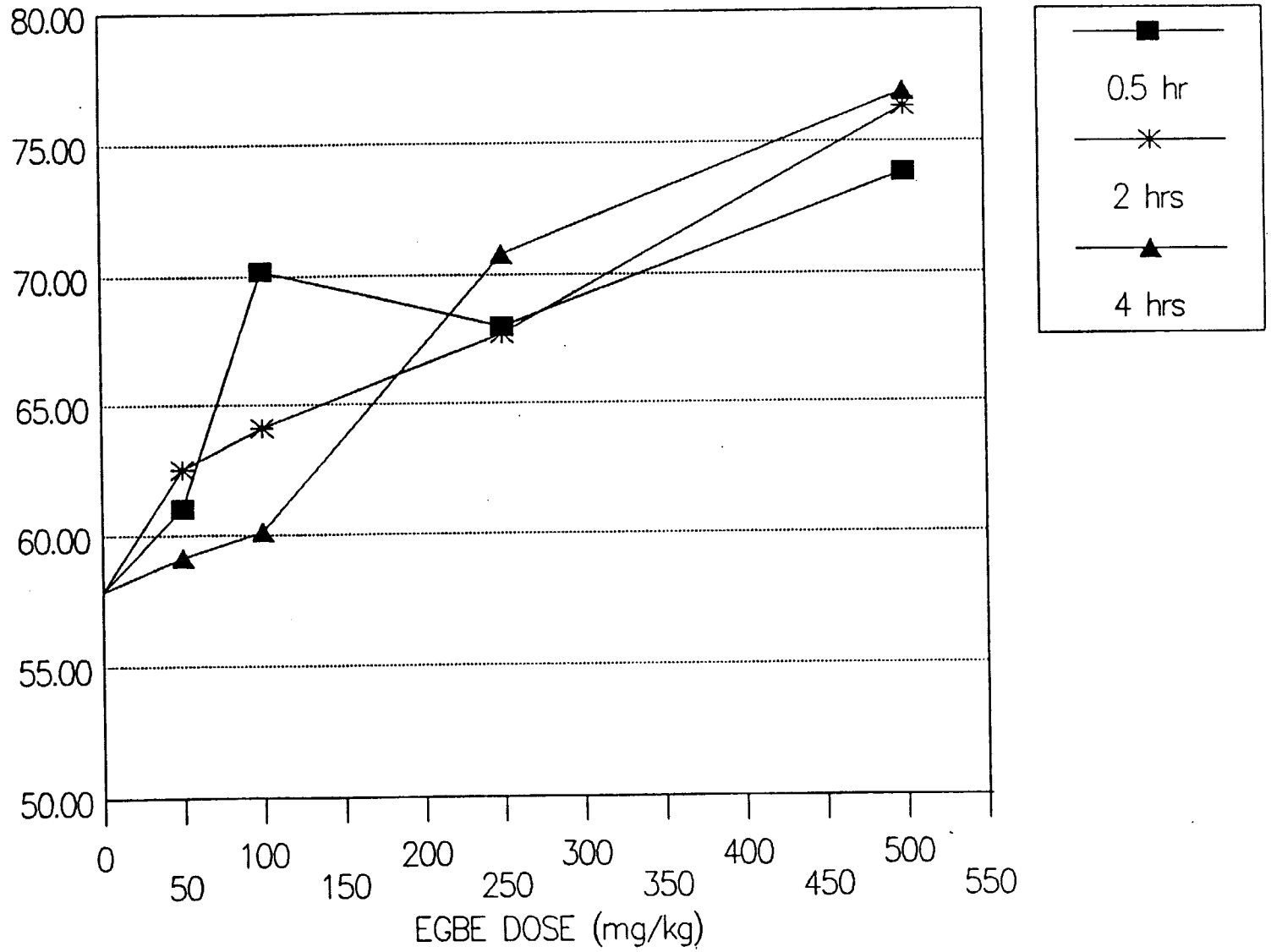
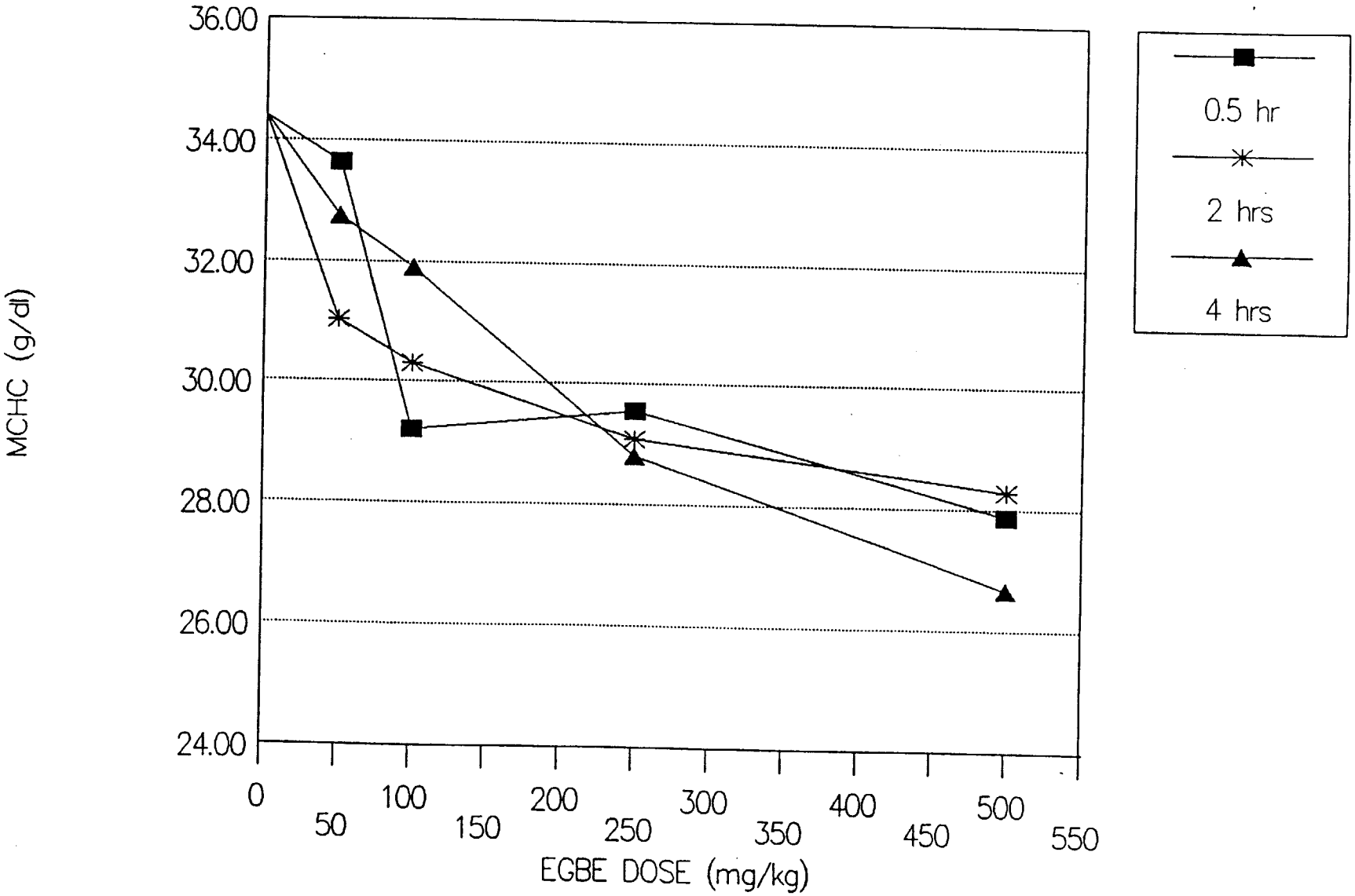


Figure 7: Dose-response relationship between the ingested dose of EGBE and the changes in the mean cell hemoglobin concentration of the circulating red blood cells in the rat given EGBE by gavage for 0.5, 2, or 4 hours.



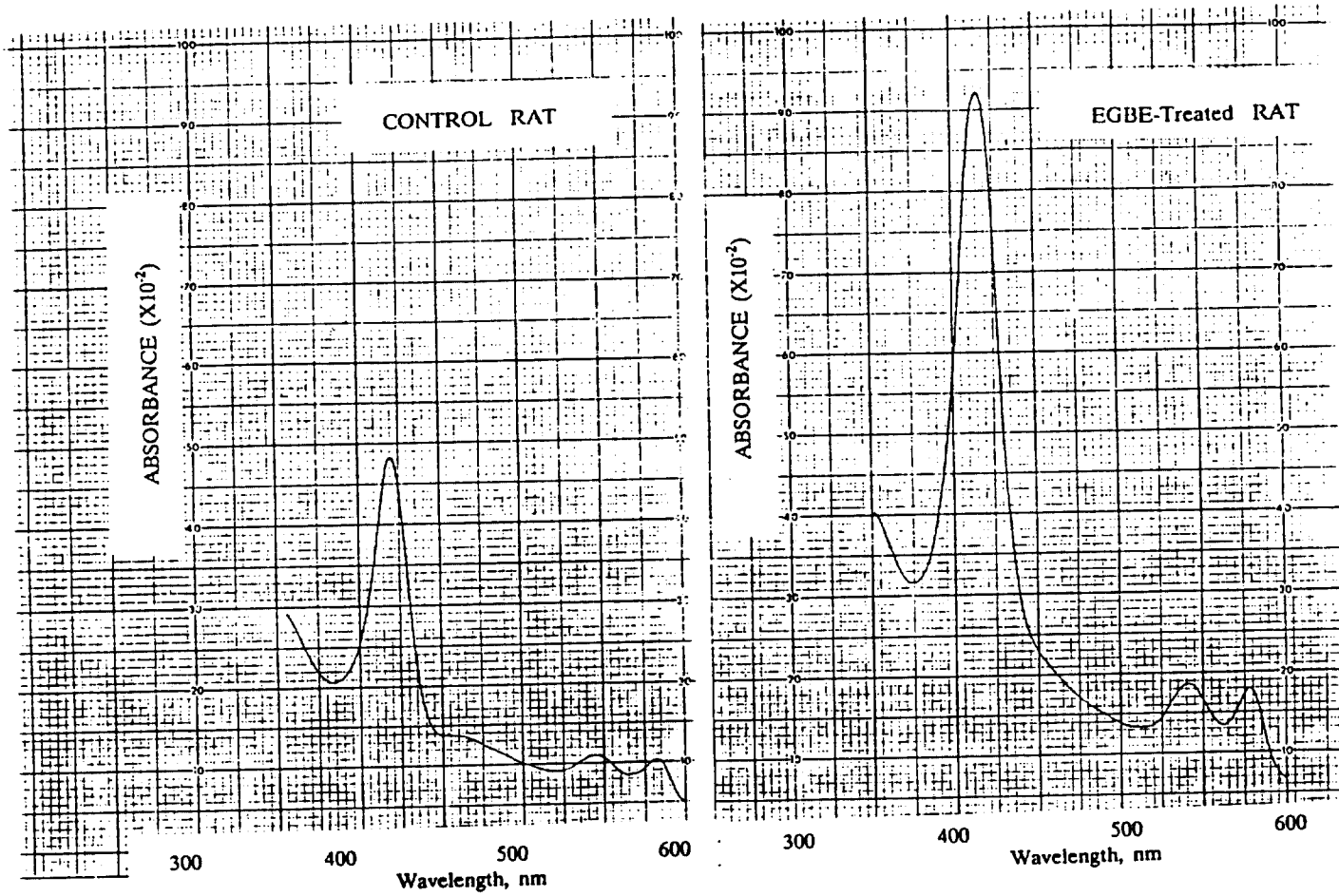


Figure 8: Illustration of the absorption spectra properties of oxyhemoglobin showing the primary peaks of interest at 415 nm, 540 nm. and 575 nm.

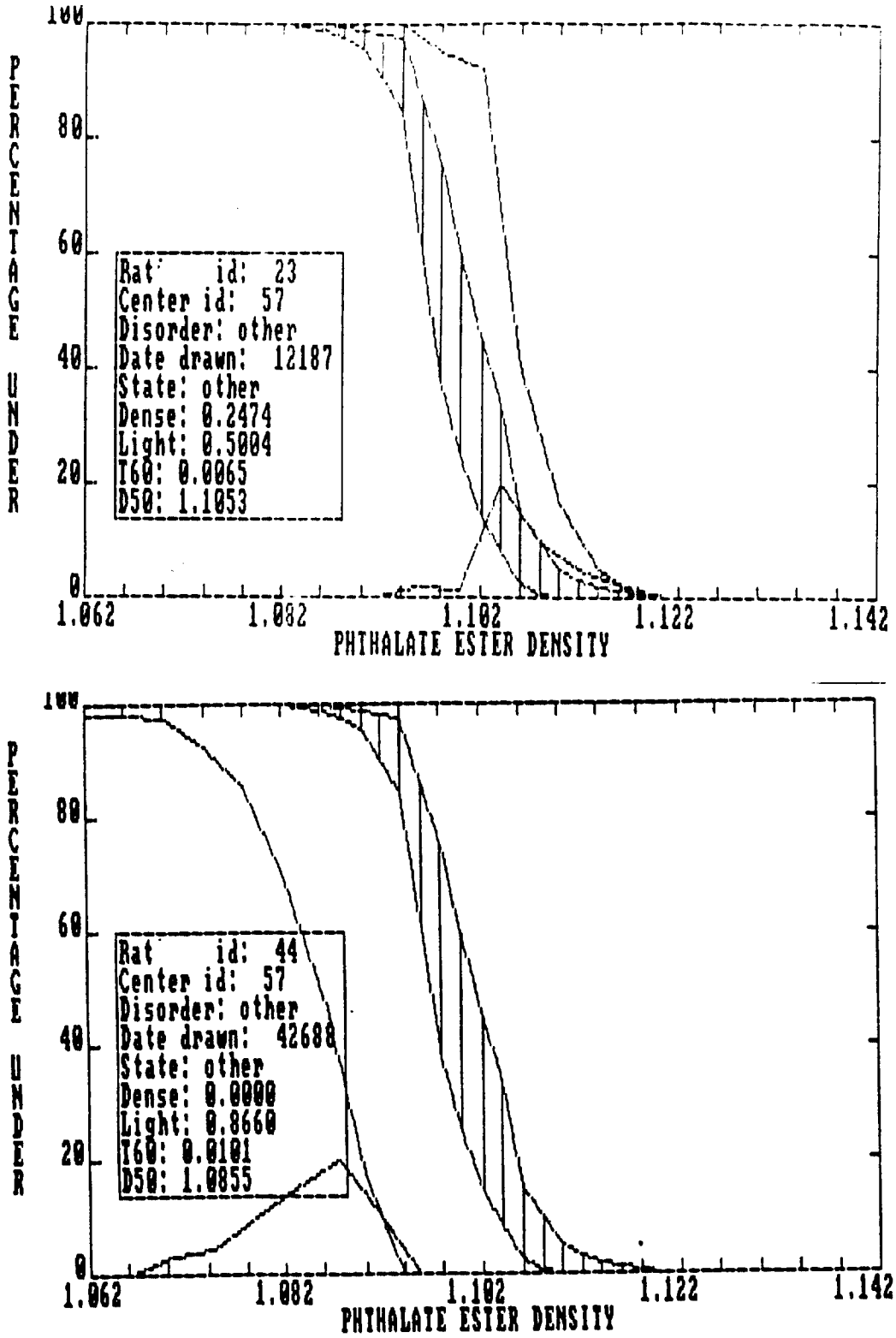
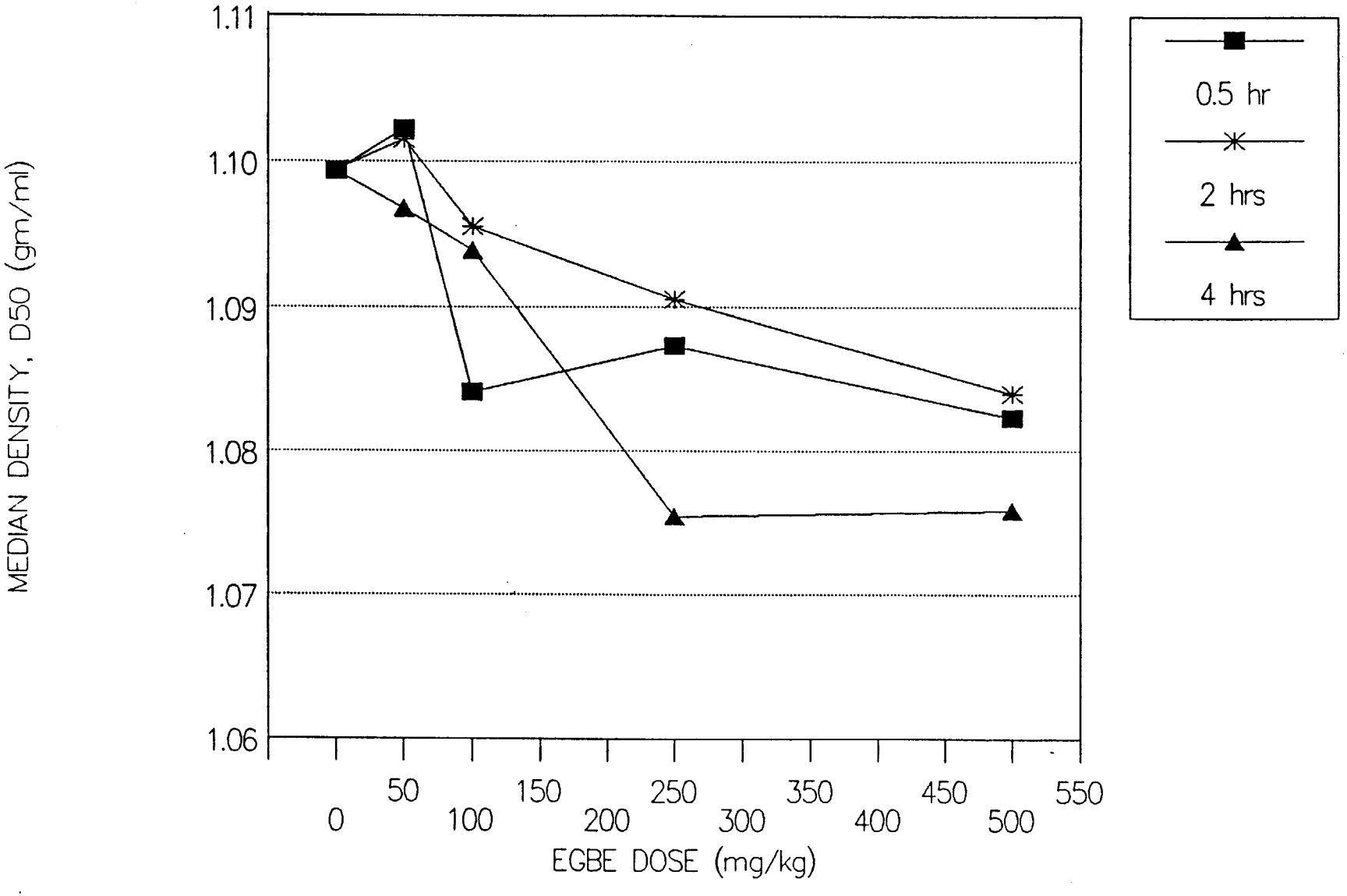


Figure 9: Erythrocyte density distribution profiles of a control rat and a EGBE-treated rat (250mg/kg, 4hr) expressed as percent of cells below the ester vs. ester density. The striped area represents 1 SD for the profile of normal human red cells which has been used to establish the reproducibility and reliability of this technique. The continuous line represents the profile for the rat red cells analyzed in duplicate. Note that the control rat red cells have a denser profile than human cells. The histogram is the first derivative of the density distribution showing the cell profile before and after exposure to EGBE and its metabolites. Also note the shift of red cells of EGBE-treated rats to the lower density range.

Figure 10: Dose-response relationship between the ingested dose of EGBE and the alterations in the median density the circulating red blood cells in the rat given EGBE by gavage for 0.5, 2, or 4 hours.



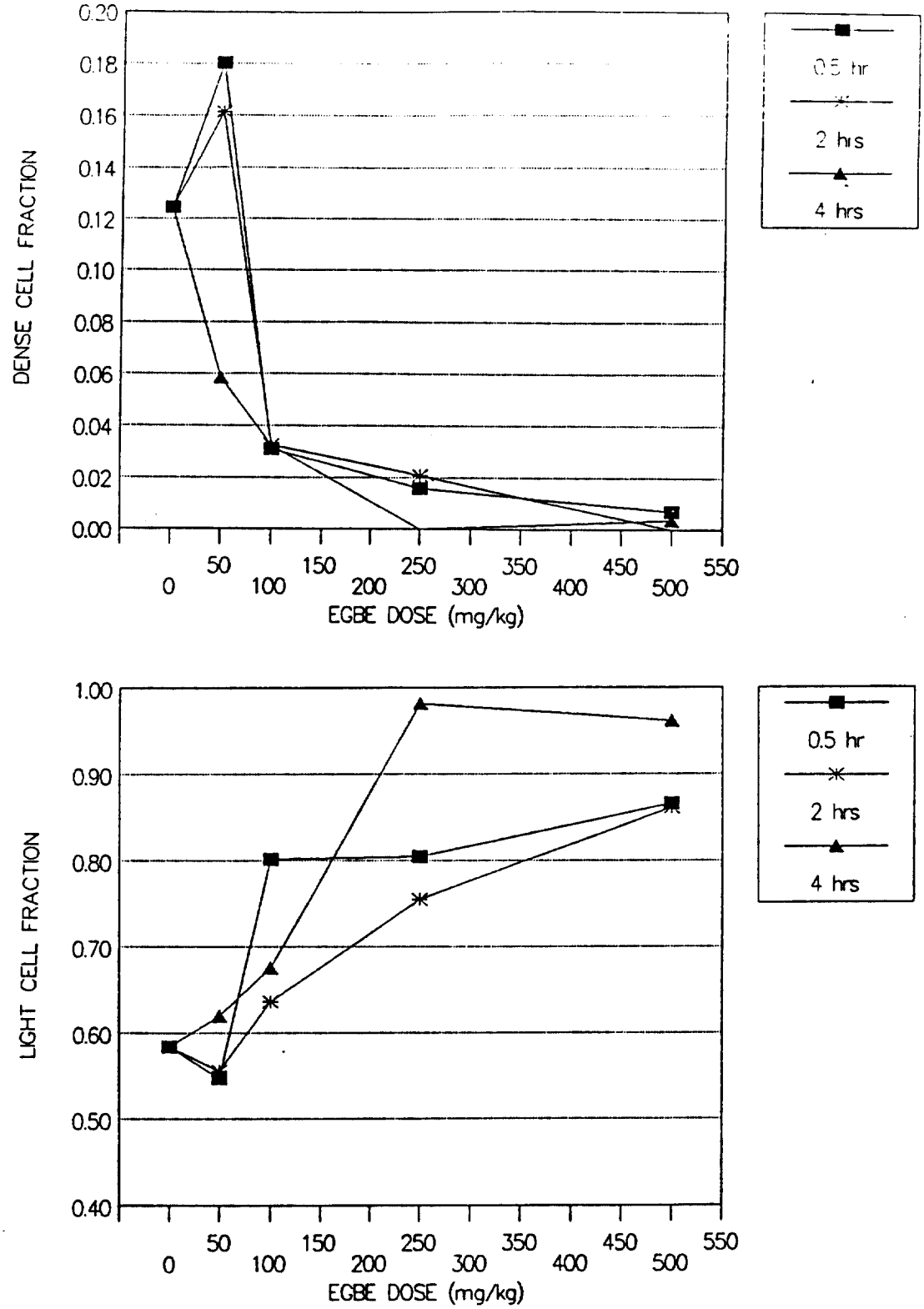


Figure 11: Dose-response relationship between the ingested dose of EGBE and the alterations in the proportions dense and light red blood cells circulating in the rat given EGBE by gavage for 0.5, 2, or 4 hours.

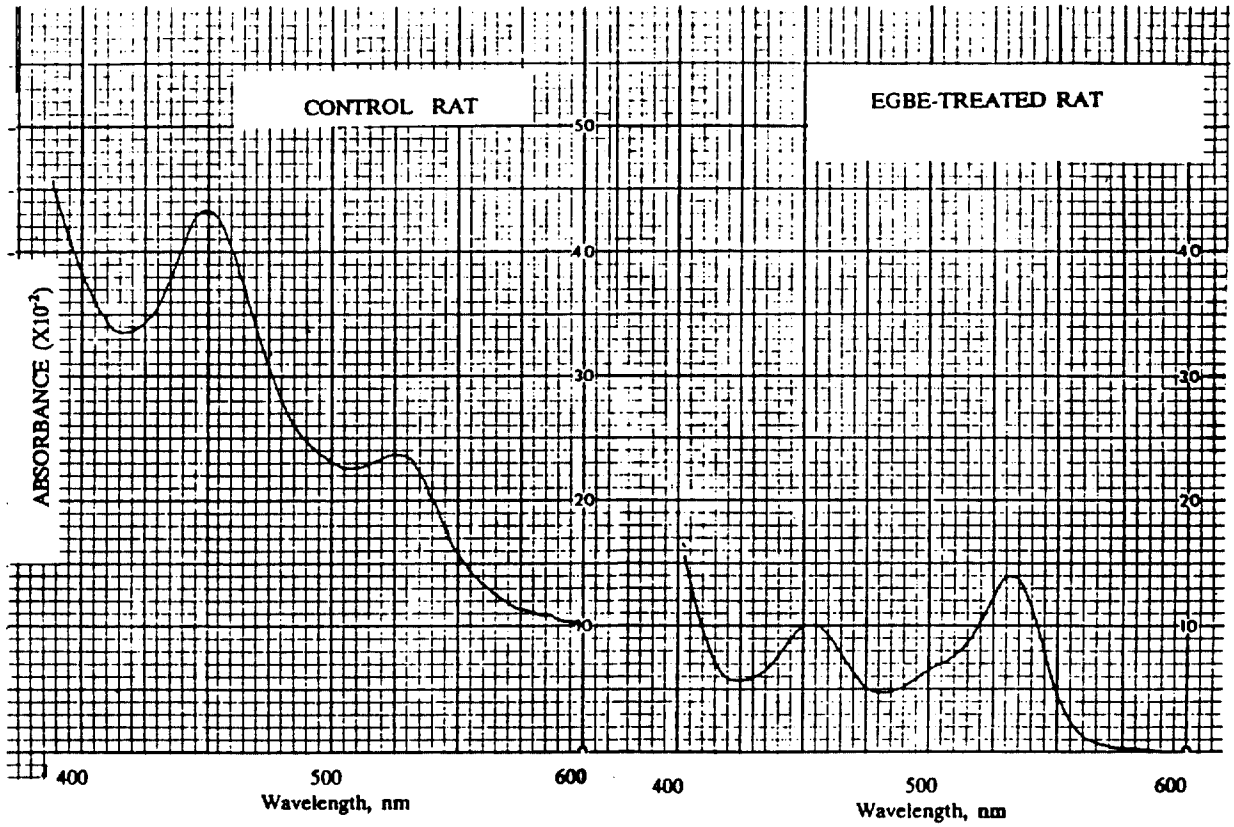


Figure 12: Illustration of the absorption spectra properties of malonyldialdehyde and thiobarbituric acid reaction complexes extracted from erythrocytes of a control and EGBE-treated rat showing the maximum peak of interest at 532 nm.

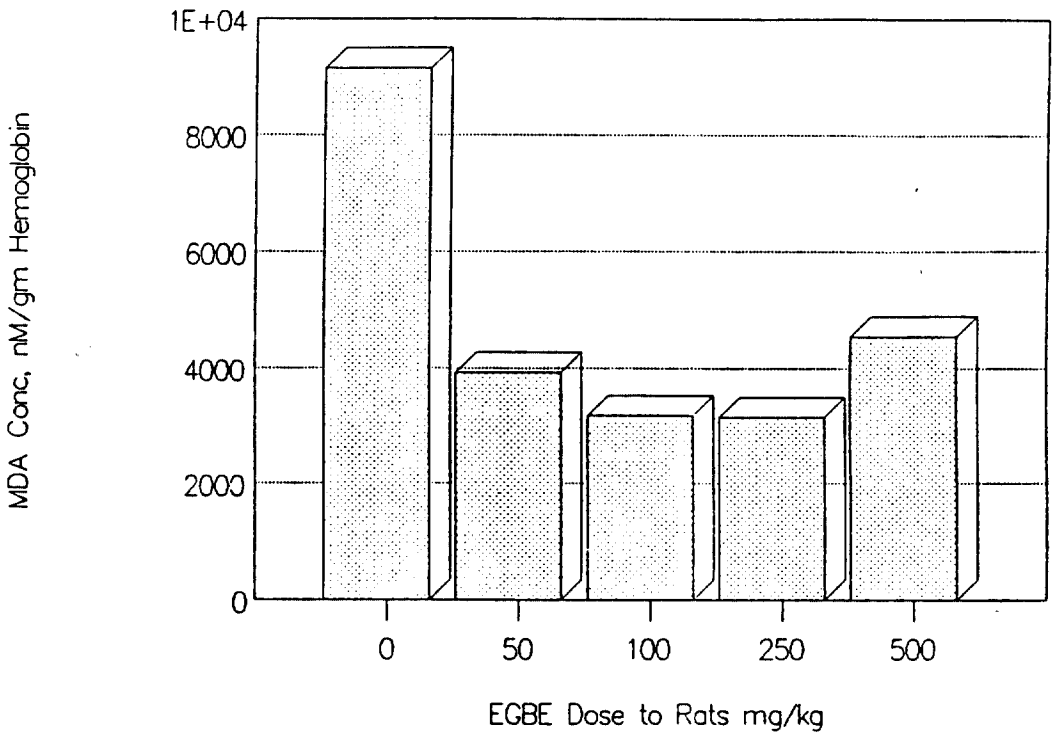


Figure 13: Dose-response relationship between the ingested dose of EGBE and the concentration of malonyldialdehyde in red blood cells circulating in the rat given EGBE by gavage for 0.5, 2, or 4 hours prior to sampling blood.

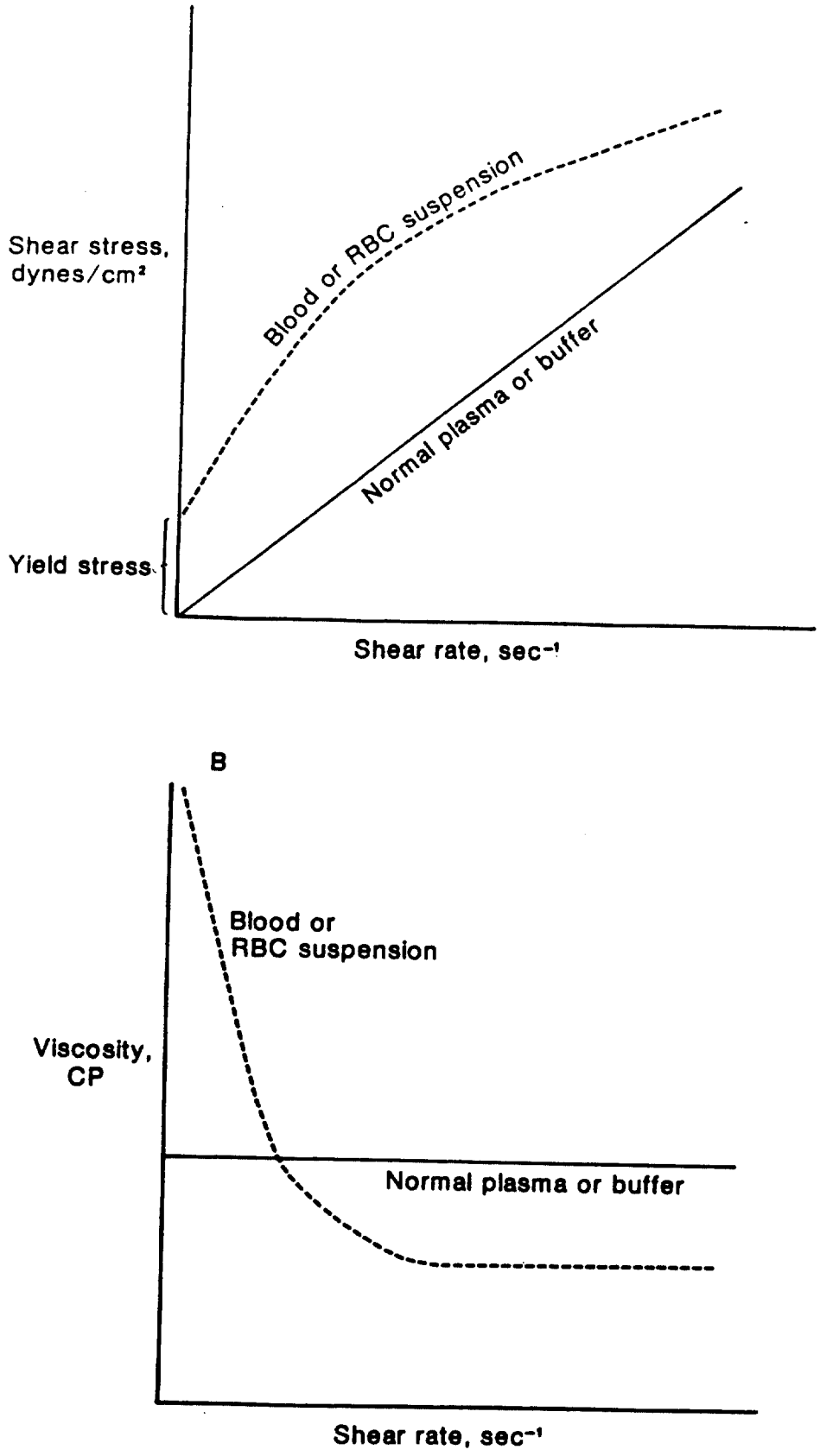


Figure 14: Illustration of the relationship between (A) shear stress and shear rate and (B) coefficient of viscosity and shear rate for Newtonian and non-Newtonian fluids (From Kurantsin-Mills, 1988).

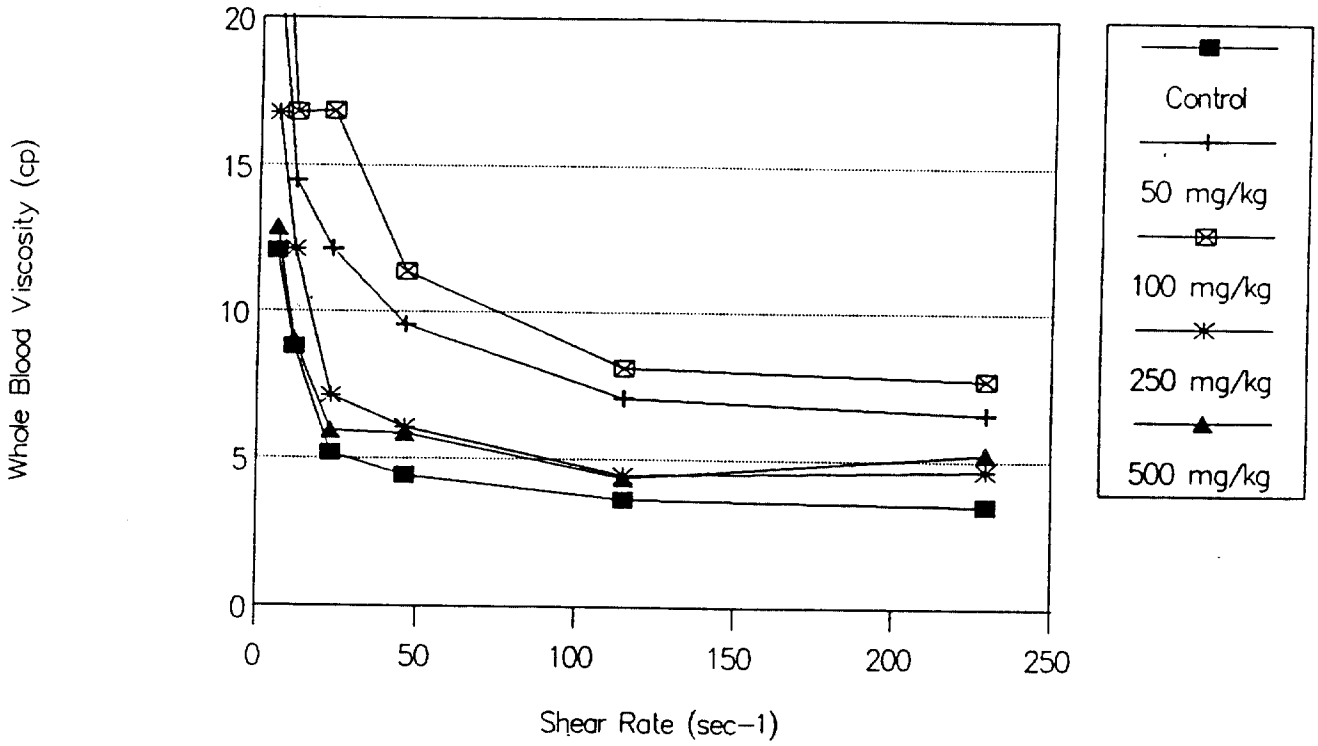


Figure 15: A plot of the relationship between coefficient of viscosity and shear rate for control rat blood and EGBE-treated (50 to 250mg/kg) rat blood. Hct = 40%; Temp. 37°C. Note that the curve for 500mg/kg dose is just below the curve for 250mg/kg. SD bars omitted for clarity.

TABLE 1: SUMMARY OF PUBLISHED STUDIES OF EGBE HEMATOXICITY

AUTHOR	YEAR	TEST ANIMAL	EXPOSURES ROUTE	DOSE	TIME	NO	TYPE	HEMATOLOGIC EFFECTS (ppm)
Werner	1943	Mice	I	390-1200 (range)	7 hrs 32	1 exp. 1 exp.	A A	- hemoglobinuria at ≥ 600 ppm - follicular phagocytosis in spleen
Werner	1943	Rats	I	135 320	7 7	x 5d x 5wks x 5d x 5wks	SC SC	- Hgb 12.5 - 11; no retic response - Hgb 12.5 - 9.5 gms 1st week with - retic response; recovery at wks 4-6 - elevated juvenile WBCs both groups
Werner	1943	Dogs	I	415	7 x 5d x 12wks		SC	- 10 % Hct, max wk 10 - no retic response seen - RBC microcytosis, hypochromia - SI ^ RBC osm fragility - no ^ WBC
Carpenter	1956	Mice	I	112	30,60,90d		SC	- ^ RBC fragility at 30, 60, and 90 days
			I	203	30,60,90d		SC	- ^ RBC fragility, hemoglobinuria (all)
			I	400	30,60,90d		SC	- ^ RBC fragility, hemoglobinuria
		Rats(F)	I	432	2hrs, 8hrs		A	- in vivo hemolysis @ 2hrs, severe at 8hr
RBC,		Rats(F)	I	200	7hrs x 9d		SC	- RBC frag. ^ during inhalation at day 4 -50% 25% Hgb
		Rats	O	0.3-3.8 gm/kg			A	- hemoglobinuria in male at 3.0 gm/kg, females at 1.5 gm/kg
		Rabbits	S	0.5-2 ml/kg x 24 hrs			A	- ^ RBC frag; 3% RBC
		Rats	IV					- hemolytic at all conc. > 3%
		Rats	I	54-432	7hrs x 5d x 6wks		SC	- ^ RBC frag. at all conc; hgburia at conc > 200 ppm
		G.Pigs	I	54-494	7hrs x 5d x 6wks		SC	- no v RBC fragility or Hgburia
		Dogs	I	385-617	13.5hrs - 28d		A&SC	- Females died at 617 ppm (13^0) and 8th day @ 385. Males died at 28 days - males showed ^ RBC frag at 7 days, returned to normal by 27 days - ^ WBC in 385 ppm exp dogs

TABLE 1 (continued)

AUTHOR (Ref)	YEAR	TEST ANIMAL	EXPOSURES ROUTE	DOSE (ppm)	TIME	NO	TYPE	HEMATOLGIC EFFECTS
Carpenter (6)	1956	Dogs	I	200	31d		SC	- s1 ^ RBC frag; ^ WBC; s1 Hgb
(cont'd)		Dogs	I	100	90d		C	- WBC ^ x 2; Hct 43 __ 34% @ 90 days
		Monkeys	I	100	90d		SC	- ^ RBC osm frag female > male - v RBC but return to normal by day 90
		Monkeys	I	210	30d		SC	- ^ RBC frag 4th day; at day 30 Hgb and RBC v by 50% (animals had TB)
		Human	I	113	4hrs	once	A	- no in RBC fragility, Hgb
		Human	I	195	8hrs	once	A	- no in RBC fragility, Hgb - limited by non-hematol Sx - human subjects excreted butoxy-acetic acid
Homan (7)	1979	Rabbits	S	2% - 100	6 hrs	x 9d	SC	- 100% EGBE caused hemoglobinuria - v Hct, ^ RBC frag. by day 2 - 5 females with Hgburia at day 9 @ 50% - v RBC, Hgb in females at day 9 @ 50% - no hematol. effects @ 25% or less
Will Res (8) Labs	1982	Rabbits	S	2.8 - 42.8	6 hrs	x 5d x 13 wks	SC	- males v RBC @ 14% at 4 wks (?) - females ? v RBC 14% at 4 wks - RBC osm. frag. nl. at 12 wks
Tyl (9)	1983	Rabbits	I	20 - 200	6 hrs	x 13d	SC	- no change in RBC, Hgb, Hct, osm. frag. or indices
		Rats	I	20 - 200	6 hrs	x 9d	SC	- v RBC at 100 and 200 ppm - hemoglobinuria
Dodd (10)	1983	Rats	I	200 - 850	4 hrs		A	- LC_{50} 450; hemoglobinuria
			I	25 - 250	6 hrs	x 9d	SC	- v RBC, Hgb, ^ Retic. at 86 + 245 ppm - values returned to nl. by day 14
			I	10 - 75	6 hrs	x 5d x13wks	SC	- 5% v RBC and Hgb, ^ MCH only at 77 ppm

Abbreviations: I = inhalation; S = skin; O = oral; IV = intravenous; A = acute; SC = subchronic; C = chronic

Table 2: Summary Statistics of Erythrocyte Indices as a of Function of EGBE Dose.

INDEX	CONTROL	50 mg/kg	100 mg/kg	250 mg/kg	500 mg/kg	F-STAT.	p-VALUE	FISHER'S LSD
MCV	57.8 ± 0.8	61.0 ± 1.3	66.1 ± 1.3	68.4 ± 1.2	75.9 ± 1.2	41.69	0.0001**	abcdefgij
MCHC	34.4 ± 0.4	32.7 ± 0.7	30.1 ± 0.7	29.1 ± 0.6	27.9 ± 0.6	22.33	0.0001**	abcdefgij
HCT	36.3 ± 0.9	35.7 ± 1.4	42.6 ± 1.3	38.2 ± 1.3	40.1 ± 1.3	5.15	0.0007**	bdegh
HgB	12.5 ± 0.2	11.7 ± 0.4	12.8 ± 0.4	11.1 ± 0.3	10.9 ± 0.3	6.65	0.0001**	cdehi
MCH	19.9 ± 0.3	20.1 ± 0.4	20.0 ± 0.4	19.8 ± 0.4	21.0 ± 0.4	2.06	0.0899	
RDW	13.4 ± 0.3	14.9 ± 0.5	13.1 ± 0.4	13.8 ± 0.4	14.7 ± 0.4	4.01	0.0042**	adei
RBC	6.3 ± 0.01	5.9 ± 0.2	6.4 ± 0.2	5.6 ± 0.2	5.3 ± 0.2	5.84	0.00002**	cdghij
HDW	2.0 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.7 ± 0.1	2.9 ± 0.1	29.68	0.0001**	cdfghij

Comments on Fisher's LSD:
control vs 50 mg = a, control vs 100 mg = b,
control vs 250 mg = c, control vs 500 mg = d,
50 mg vs 100 mg = e, 50 mg vs 250 mg = f,
50 mg vs 500 mg = g, 100 mg vs 250 mg = h,
100 mg vs 500 mg = i, 250 mg vs 500 mg = j.

Table 3: Statistics of Erythrocyte Indices of Control and EGBE-Treated Rats

Dose	MCV (fl) N	MCV (fl) MEAN ± SEM	RDW (%) N	RDW (%) MEAN ± SEM	MCHC (g/dl) N	MCHC (g/dl) MEAN ± SEM	HDW (g/dl) N	HDW (g/dl) MEAN ± SEM
Control	48	57.88 ± 0.40	45	13.37 ± 0.28	48	34.40 ± 0.15	45	2.01 ± 0.0

Dose	HCT (%) N	HCT (%) MEAN ± SEM	HGB (g/dl) N	HGB (g/dl) MEAN ± SEM	RBC (E6/ul) N	RBC (E6/ul) MEAN ± SEM	MCH (pg) N	MCH (pg) MEAN ± SEM
Control	48	36.27 ± 0.60	48	12.48 ± 0.22	48	6.32 ± 0.12	48	19.90 ± 0.1

Time	Dose	MCV (fl) N	MCV (fl) MEAN ± SEM	RDW (%) N	RDW (%) MEAN ± SEM	MCHC (g/dl) N	MCHC (g/dl) MEAN ± SEM	HDW (g/dl) N	HDW (g/dl) MEAN ± SE
0.5 hr	50mg/kg	10	61.04 ± 0.73	8	13.28 ± 0.30	10	33.64 ± 0.30	8	2.06 ± 0.07
	100mg/kg	11	70.96 ± 3.26	9	12.43 ± 0.20	11	30.12 ± 1.38	9	1.82 ± 0.1
	250mg/kg	5	68.00 ± 4.77	5	12.62 ± 0.51	5	29.56 ± 1.47	5	2.23 ± 0.11
	500mg/kg	5	73.80 ± 3.76	5	12.98 ± 0.64	5	27.88 ± 1.27	5	2.23 ± 0.11
	F-stat		3.100		NS		3.47		3.68
	P-VCGM		0.043		NS		0.029		0.027

Time	Dose	HCT (%) N	HCT (%) MEAN ± SEM	HGB (g/dl) N	HGB (g/dl) MEAN ± SEM	RBC (E6/ul) N	RBC (E6/ul) MEAN ± SEM	MCH (pg) N	MCH (pg) MEAN ± SE
0.5 hr	50mg/kg	10	34.10 ± 2.67	10	11.49 ± 0.94	10	5.58 ± 0.43	10	20.56 ± 0.1
	100mg/kg	11	42.80 ± 1.82	11	12.70 ± 0.41	11	6.06 ± 0.17	11	20.90 ± 0.2
	250mg/kg	5	38.74 ± 2.40	5	11.26 ± 0.11	5	5.71 ± 0.07	5	19.78 ± 0.4
	500mg/kg	5	44.44 ± 2.30	5	12.28 ± 0.25	5	6.03 ± 0.18	5	20.36 ± 0.2
	F-stat		3.480		NS		NS		NS
	P-VCGM		0.029		NS		NS		NS

P-VCGM = p value comparing group means

Table 3: Statistics of Erythrocyte Indices of Control and EGBE-Treated Rats (continued)

Time	Dose	MCV (fl) N	MCV (fl) MEAN ± SEM	RDW (%) N	RDW (%) MEAN ± SEM	MCHC (g/dl) N	MCHC (g/dl) MEAN ± SEM	HDW (g/dl) N	HDW (g/dl) MEAN ± SEM
2 Hrs	50mg/kg	6	62.53 ± 2.88	6	17.58 ± 1.37	6	31.03 ± 1.13	6	2.32 ± 0.07
	100mg/kg	7	64.11 ± 1.78	7	14.20 ± 0.86	7	30.30 ± 1.77	7	2.51 ± 0.09
	250mg/kg	15	67.69 ± 1.94	15	14.17 ± 0.40	15	29.09 ± 0.62	15	2.69 ± 0.12
	500mg/kg	15	76.30 ± 1.05	15	15.34 ± 0.32	15	28.29 ± 1.65	15	3.10 ± 0.13
	F-stat		10.390		4.550		NS		5.73
	P-VCGM		0.0001		0.0080		NS		0.0029

Time	Dose	HCT (%) N	HCT (%) MEAN ± SEM	HGB (g/dl) N	HGB (g/dl) MEAN ± SEM	RBC (E6/ul) N	RBC (E6/ul) MEAN ± SEM	MCH (pg) N	MCH (pg) MEAN ± SEM
2 hrs	50mg/kg	6	39.23 ± 2.71	6	12.12 ± 0.82	6	6.39 ± 0.60	6	19.23 ± 0.48
	100mg/kg	7	43.54 ± 3.80	7	12.87 ± 0.77	7	6.80 ± 0.56	7	19.27 ± 0.81
	250mg/kg	15	38.64 ± 1.36	15	11.24 ± 0.44	15	5.80 ± 0.30	15	19.56 ± 0.35
	500mg/kg	15	38.11 ± 2.44	15	10.39 ± 0.44	15	4.98 ± 0.30	15	21.49 ± 1.15
	F-stat		NS		3.11		3.62		NS
	P-VCGM		NS		0.037		0.021		NS

P-VCGM = p value comparing group means

Table 3: Statistics of Erythrocyte Indices of Control and EGBE-Treated Rats (continued)

Time	Dose	MCV (fl) N	MCV (fl) MEAN ± SEM	RDW (%) N	RDW (%) MEAN ± SEM	MCHC (g/dl) N	MCHC (g/dl) MEAN ± SEM	HDW (g/dl) N	HDW (g/dl) MEAN ± SEM
4 Hrs	50mg/kg	5	59.14 ± 0.68	5	12.82 ± 0.30	5	32.74 ± 0.21	5	2.17 ± 0.08
	100mg/kg	5	60.12 ± 0.49	5	12.68 ± 0.21	5	31.90 ± 0.10	5	1.92 ± 0.08
	250mg/kg	5	70.76 ± 2.60	5	14.08 ± 0.64	5	28.80 ± 0.81	5	3.03 ± 0.13
	500mg/kg	5	76.90 ± 3.83	5	14.72 ± 0.29	5	26.68 ± 0.77	5	2.98 ± 0.11
	F-stat		10.63		5.04		19.25		23.63
	P-VCGM		0.0004		0.0120		0.0001		0.0001

Time	Dose	HCT (%) N	HCT (%) MEAN ± SEM	HGB (g/dl) N	HGB (g/dl) MEAN ± SEM	RBC (E6/ul) N	RBC (E6/ul) MEAN ± SEM	MCH (pg) N	MCH (pg) MEAN ± SEM
4 hrs	50mg/kg	5	34.82 ± 1.59	5	11.42 ± 0.59	5	5.90 ± 0.33	5	19.40 ± 0.15
	100mg/kg	5	40.62 ± 0.49	5	12.96 ± 0.13	5	6.76 ± 0.09	5	19.16 ± 0.16
	250mg/kg	5	36.20 ± 1.75	5	10.42 ± 0.53	5	5.17 ± 0.35	5	20.28 ± 0.47
	500mg/kg	5	41.80 ± 2.33	5	11.08 ± 0.34	5	5.43 ± 0.08	5	20.38 ± 0.50
	F-stat		3.24		4.87		6.45		NS
	P-VCGM		0.0004		0.0136		0.0045		NS

P-VCGM = p value comparing group means

Table 4: Plasma and urine hemoglobin concentrations of control and rats treated with different doses of EGBE.

EGBE (mg/kg)	PLASMA [Hgb] g/dl 415 nm n	MEAN ± SD	[Hgb] g/dl 540 nm n	MEAN ± SD	URINE [Hgb] g/dl 415 nm n	MEAN ± SD	[Hgb] g/dL 540 nm n	MEAN ± SD
Control	24	0.439 ± 0.161	24	0.028 ± 0.022	8	0.064 ± 0.081 b	8	0.016 ± 0.010
50 mg	5	0.0553 ± 0.122	5	0.025 ± 0.011	4	0.094 ± 0.083 b	4	0.016 ± 0.003
100 mg	7	0.359 ± 0.191	7	0.031 ± 0.021	5	0.136 ± 0.127 b	5	0.017 ± 0.008
250 mg	6	0.520 ± 0.122	6	0.056 ± 0.047	3	0.373 ± 0.215 a	3	0.011 ± 0.009
500 mg	4	0.495 ± 0.026	4	0.026 ± 0.009	4	0.135 ± 0.066 b	4	0.003 ± 0.005
G.M.C.								
F-STAT	1.640		1.690		4.35		2.29	
P-value	0.182		0.172		0.012*		0.098	

MEANS WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT FROM EACH OTHER AT THE 0.05 LEVEL OF SIGNIFICANCE.
* $P<0.05$

Table 5: Red Cell Density Indices of control and EGBE-treated Rats.

TIME	50mg/kg		100mg/kg		250mg/kg		500mg/kg		P-VALUE C.
D50:	n	MEAN + SE	n	MEAN + SE	n	MEAN + SE	n	MEAN + SE	GROUP MEANS
0.5 hr.	8	1.0905 ± 0.0324	27	1.0814 ± 0.0169	8	1.0873 ± 0.0063	8	1.0823 ± 0.0089	0.595
2 hrs.	9	1.1016 ± 0.0025 a	8	1.0955 ± 0.00014b	10	1.0873 ± 0.0044	8	1.0840 ± 0.0042d	<0.001**
4 hrs.	6	1.0967 ± 0.0019 b	6	1.0939 ± 0.00340b	2	1.0755 ± 0.0004	6	1.0758 ± 0.0032a	<0.001**
T60:									
0.5 hr.	8	0.0052 ± 0.0052	27	0.0059 ± 0.0020	8	0.0089 ± 0.0031	8	0.0086 ± 0.0026	<0.001**
2 hrs.	9	0.0051 ± 0.0012	8	0.0051 ± 0.0006	10	0.0088 ± 0.0038	8	0.0119 ± 0.0037	<0.001**
4 hrs.	6	0.0049 ± 0.0090	6	0.0049 ± 0.0012	2	0.0116 ± 0.0002	6	0.0147 ± 0.0037	<0.001**
DCF:									
0.5 hr.	8	0.1802 ± 0.0223	27	0.0299 ± 0.0686	8	0.0159 ± 0.0254	8	0.0072 ± 0.0128	<0.001**
2 hrs.	9	0.1622 ± 0.0521 a	8	0.0325 ± 0.0223bc	10	0.0196 ± 0.0249d	8	0.0000 ± 0.0000cd	<0.001**
4 hrs.	6	0.0583 ± 0.0477	6	0.0323 ± 0.0263	2	0.9815 ± 0.0262	6	0.0036 ± 0.0056	<0.030*
LCF:									
0.5 hr.	8	0.5469 ± 0.0129	27	0.8022 ± 0.1712	8	0.8039 ± 0.1345	8	0.8590 ± 0.1405	<0.001**
2 hrs.	9	0.5553 ± 0.0174	8	0.6368 ± 0.0248	10	0.7287 ± 0.0961	8	0.8606 ± 0.0752	<0.001**
4 hrs.	6	0.6198 ± 0.0299	6	0.6751 ± 0.0712	2	0.9815 ± 0.0262	6	0.9611 ± 0.0380	<0.001**

MEANS WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT AT THE 0.05 LEVEL

* $P<0.05$

** $P<0.01$

Table 6: Red Cell Adenosine Triphosphate (ATP) Concentrations of control and EGBE-treated rats.

VARIABLE	CONTROL		50 MG		100 MG		250 MG		500 MG		P-Value
	n	MEAN ± SD	n	MEAN ± SD	n	MEAN ± SD	n	MEAN ± SD	n	MEAN ± SD	(FSTAT)
MCV	24	55.03 ± 8.02a	6	62.53 ± 7.74b	5	62.74 ± 4.78b	8	67.11 ± 7.26b	15	74.83 ± 5.56c	0.0001**
ATP	24	0.94 ± 0.30	7	1.00 ± 0.32	7	1.18 ± 0.46	11	1.15 ± 0.39	15	1.16 ± 0.25	0.1801

MEANS WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT FROM EACH OTHER AT P-VALUE LESS THAN 0.05.

Table 7:
Red Blood Cell Malonyldialdehyde (MDA) Concentration in control and EGBE-treated Rats

		EGBE, mg/kg (Time 2hrs)				
		0 mg/kg	50 mg/kg	100 mg/kg	250 mg/kg	500 mg/kg
MDA (nm/ gm Hgb)	n	6	7	7	7	7
	mean	9157.12	3924.63	3185.31	3150.39	4543.70
	Std	1297.80	1346.32	320.24	508.82	1964.72

Table 8:
Plasma and Whole Blood Viscosity of the Control and EGBE-treated Rats

EGBE, mg/kg (Time 2hrs)		Plasma Viscosity (cp)	Blood Viscosity (cp)					
			5.75	11.5	23	46	115	230
0	n	7	7	7	7	7	7	7
	mean	1.45	12.07	8.80	5.19	4.44	3.63	3.50
	std	0.27	2.91	2.32	1.79	1.05	0.51	0.54
50	n	2	2	2	2	2	2	2
	mean	3.165	21.05	14.47	12.15	9.55	7.08	6.585
	std	0.125	0.25	0.07	0.35	0.15	0.04	0.085
100	n	2	2	2	2	2	2	2
	mean	3.375	26.1	16.8	16.85	11.38	8.09	7.73
	std	0.015	0.9	1.54	0.05	0.22	0.29	0.06
250	n	3	3	3	3	3	3	3
	mean	2.25	16.77	12.15	7.13	6.07	4.45	4.66
	std	0.20	0.74	1.08	0.69	0.27	0.15	0.28
500	n	4	4	4	4	4	4	4
	mean	2.50	12.86	9.04	5.94	5.85	4.36	5.25
	std	0.42	1.21	0.90	0.52	0.85	0.35	0.25

Table 9: Plasma hemoglobin concentrations of sheared and unsheared blood of control and EGBE-treated rats. Whole blood was sheared in a viscometer at 230/sec as described in the Methods.

	UNSHEARED BLOOD		SHEARED BLOOD	
EGBE, mg/kg	[Hgb] g/dL 415 nm	[Hgb] g/dL 540 nm	[Hgb] g/dL 415 nm	[Hgb] g/dL 540 nm
CONTROL O				
N	7	7	4	4
MEAN ± SD	0.504 ± 0.099	0.023 ± 0.005	0.563 ± 0.025b	0.029 ± 0.006b
250 mg (2 hrs.)				
N	6	6	6	6
MEAN ± SD	0.520 ± 0.122	0.033 ± 0.011	0.621 ± 0.029b	0.049 ± 0.009a
500 mg (2 hrs.)				
N	4	4	7	7
MEAN ± SD	0.495 ± 0.112	0.026 ± 0.009	0.026 ± 0.064a	0.057 ± 0.017a
F-STAT C.G.M.	0.07	2.34	7.06	6.18
P-VALUE	0.936	0.133	0.008**	0.012*

MEANS WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT FROM EACH OTHER AT THE 0.05 LEVEL OF SIGNIFICANCE.

* $P<0.05$

** $P<0.01$

Table 10: Summary Data of Red Cell Deformability Indices of Control and EGBE-treated Rats

Control Rats

Rat#	P_o	P_i	P_i/P_o	Vc	Vp	V	B
112	5.5	11.2	2.04	55.5	70.7	0.785	1.82
112	11.0	19.0	1.73	55.5	70.7	0.785	1.57
113	10.0	18.0	1.80	55.5	70.7	0.785	1.63
114	1.2	3.0	2.40	55.5	70.7	0.785	2.10

EGBE-treated Rats

Rat#	P_o	P_i	P_i/P_o	V_c	V_p	V	B
115	4.0	59.7	14.9	70.5	70.7	0.997	14.86
116	2.0	29.7	14.8	76.9	70.7	1.088	16.01
117	6.75	75.0	11.1	74.2	70.7	1.049	11.59
117	2.0	75.0	39.1	74.2	70.7	1.049	39.29
118	0.5	60.0	120	75.2	70.7	1.064	127.6

P_o initial flow of Buffer in the pore; P_i initial flow of RBCs in the pore; V_c volume of cells, MCV; V_p volume of pore; $V = V_c/V_p$
$B = 1 + [(p_i/p_o) - 1] v/h$

Triage of 8(e) Submissions

Date sent to triage: 1/8/97 **NON-CAP** (CAP)

Submission number: 12457A TSCA Inventory: (Y) N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

(ATOX) SBTOX SEN w/NEUR

Acute (blood effects)

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX GTOX

STOX/ONCO CTOX/ONCO IMMUNO (CYTO) NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

For Contractor Use Only

entire document: (0) 1 2 **pages** 1,2 **pages** _____

Notes:

Contractor reviewer : JW **Date:** 1/17/96

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ- 0992 - 12457 SEQ. A

TYPE: INT. SUPP FLWP

SUBMITTER NAME: Union Carbide Corporation

SUB. DATE: 09/22/92 OTS DATE: 09/29/92 CSRAD DATE: 06/03/95

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
0577 REFER TO CHEMICAL SCREENING
0578 CAP NOTICE

VOLUNTARY ACTIONS:
0481 NO ACTION REPORTED
0482 STUDIES PLANNED/UNDERWAY
0483 NOTIFICATION OF WORKER HISTORIES
0484 LABEL/MSDS CHANGES
0485 PROCESS/HANDLING CHANGES
0486 APP./USE DISCONTINUED
0487 PRODUCTION DISCONTINUED
0488 CONFIDENTIAL

CHEMICAL NAME: EGBE ethylene glycol monobutyl ether

CAS# 111-76-2

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
0204	MUTA (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
0213	SUB ACUTE TOX (ANIMAL)	01 02 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
0224	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
0243	CHEM/PHYS PROP	01 02 04
0244	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
0248	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER CYTOTOXICITY (IN VITRO)	01 02 04

TRIAGE DATE:

NON-CBI INVENTORY: YES / NO

CAS SR

IN TERMINI

ONGOING REVIEW: YES (DROP/REFER) / NO (CONTINUE)

REFER

SPECIES: RAT / IN VITRO

TOXICOLOGICAL CONCERN: LOW / MED / HIGH

USE:

PRODUCTION:

SUMMARY: The mechanistic processes (included erythrocyte shape, density, energy content, lipid peroxidation, dose- and time-dependent lysis) underlying the hematologic toxicity of EGBE were studied in rats receiving a single dose of 50-500 mg/kg by gavage. The result of these experiments showed that membrane and cellular changes in the red cell altered rheological properties resulting in hemolysis at all the dose levels.

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ- 6992-12457 SEQ A

TYPE: (INT) SUPP FLWP

SUBMITTER NAME: Union Carbide Corp.

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKERS/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP/USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 9-22-92 OTS DATE: 9-29-92 CSRAD DATE: 6-13-95

CHEMICAL NAME: EGBE

CAS# 111-76-2

INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04	0216	EPI/CLIN	01 02 04	0241	IMMUNO (ANIMAL)	01 02 04
0202	ONCO (ANIMAL)	01 02 04	0217	HUMAN EXPOS (PROD CONTAM)	01 02 04	0242	IMMUNO (HUMAN)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04	0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04	(0243)	CHEM/PHYS PROP	01 02 04
0204	MUTA (IN VITRO)	01 02 04	0219	HUMAN EXPOS (MONITORING)	01 02 04	0244	CLASTO (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04	0220	ECO/AQUA TOX	01 02 04	0245	CLASTO (ANIMAL)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04	0221	ENV. OCCC/REL/FATE	01 02 04	0246	CLASTO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04	0222	EMER INCI OF ENV CONTAM	01 02 04	0247	DNA DAM/REPAIR	01 02 04
0208	NEURO (HUMAN)	01 02 04	0223	RESPONSE REQEST DELAY	01 02 04	0248	PROD/USE/PROC	01 02 04
0209	NEURO (ANIMAL)	01 02 04	0224	PROD/COMP/CHEM ID	01 02 04	0251	MSDS	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04	0225	REPORTING RATIONALE	01 02 04	(0299)	OTHER Cyto in vitro	01 (02) 04
0211	CHR. TOX. (HUMAN)	01 02 04	0226	CONFIDENTIAL	01 02 04			
(0212)	ACUTE TOX. (ANIMAL)	01 (02) 04	0227	ALLERG (HUMAN)	01 02 04			
0213	SUB ACUTE TOX (ANIMAL)	01 02 04	0228	ALLERG (ANIMAL)	01 02 04			
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04	0239	METAB/PHARMACO (ANIMAL)	01 02 04			
0215	CHRONIC TOX (ANIMAL)	01 02 04	0240	METAB/PHARMACO (HUMAN)	01 02 04			

ATOX

TRIAGE DATA:

NON-CBI INVENTORY: (YES) NO IN TRAINING

ONGOING REVIEW: YES (DROP/REFER) NO (CONTINUE) REFER

SPECIES: Rat

TOXICOLOGICAL CONCERN: LOW (MED) HIGH

USE: PRODUCTION:

CAS SR

COMMENTS